The Journal of Laboratory and Clinical Medicine

VOL. 24 SEPTEMBER, 1939 NO. 12

CLINICAL AND EXPERIMENTAL

THE INFLUENCE OF VITAMIN D ON THE SERUM PHOSPHATASE ACTIVITY IN ARTHRITIS*

PAUL W. SMITH, PH.D., A. DEO KLEIN, B.S., AND IRVING E. STECK, M.D.
CHICAGO, ILL.

IN RECENT years numerous reports have indicated that the phosphatase activity of the serum may vary widely from normal values, usually in an upward direction, in bone diseases of various types. The literature has been carefully reviewed by Kay,[1] and more recently by Morris and Peden.[2] On the other hand, relatively little attention has been paid to the possibility of phosphatase activity changes in chronic arthritis of the atrophic or hypertrophic types, although they may involve osseous changes of considerable magnitude. Kay[3] reported that plasma phosphatase activity values in arthritis may vary at random within, above, or below the accepted normal range. Race[4] did not report values significantly different from normal ones.

During the past few years we have had available for study a large number of cases of chronic arthritis from which all but the atrophic and hypertrophic types have been excluded by careful diagnosis. At the start of this investigation a large proportion of these patients had been taking some form of vitamin D in massive doses (150,000 to 400,000 units per day), with results comparable to those previously described.[5]

We were interested in finding whether the serum phosphatase activity differs in the two types of the disease, and the possible effects upon it of prolonged administration of large amounts of vitamin D.

In all, 29 persons were studied, divided into groups as follows: Group A, consisting of 18 atrophic and a subgroup of 5 hypertrophic patients who had

*From the Departments of Physiology and Medicine, College of Medicine, University of Illinois.

A part of the expenses of this investigation was borne by a grant from the Nutrition Research Laboratories.

Received for publication, January 25, 1939.

been receiving vitamin D prior to the first phosphatase observations, which were made at intervals of approximately one month with occasional omissions when patients did not report regularly. The observations extended over periods of three to nine months. Group B, 6 atrophic patients upon whom were made two phosphatase determinations, one week apart, after which viosterol administration was started. Observations then followed at irregular intervals of one week to one month, extending over five months while vitamin D administration continued.

In addition to the foregoing, a group of normal subjects, 19 in all, were studied, to establish a normal range of individual variations as well as a normal group range with the methods of analysis used.

TABLE I

PHOSPHATASE ACTIVITY, BODANSKY UNITS, NORMAL YOUNG MALES

SUBJECT	AGE	BLOOD PRESSURE	FIRST MONTH	SECOND MONTH	THIRD MONTH	FOURTH MONTH	VARIATION GREATER THAN 1 UNIT	AVERAGES
R. C.	27	125/70	2.20	2.90	3.20		1.00	2.79
H. DeC.	49	210/120	4.00	3.25	3.82			3.69
P. K.	26	124/70	3.02	2.90	1.98		1.04	2.63
B. A.	23	119/74	2.01	3.42	1.98		1.44	2.47
W.	24	127/84	4.29	4.02	3.86			4.06
K.	22	123/75	2.54	2.21	2.76			2.50
R. L.	17	120/75	4.29	3.96	4.10			4.12 (Highest)
L. B.	28	136/83	1.77	2.90	2.36	2.0	1.33	2.26
G.	21	121/78	4.20	3.84	3.60	4.19		3.96
D. R.	23	118/70	3.56	3.47	3.51	3.14		3.42
R. L.	22	110/90	2.92	3.54	3.54			3.33
D. K.	23	135/80	3.24	3.90	2.91	3.51		3.39
Y. T.	21	129/86	3.28	2.90	3.81	3.50		3.37
C.	20	126/78	4.41	3.81	4.21			4.11
C. M.	26	129/83	2.95	2.61	2.05			2.54
J. S.	22	123/78	1.98	3.64	2.76		1.66	2.79
B.	24	130/85	3.96	4.25	4.11			4.11
D. V.	22	126/90	2.01	1.90	1.90			1.94 (Lowest)
H. C.	24	120/78	3.52	2.89	0.40		1.11	3.47
							Average	3.21

All phosphatase determinations were done by the method of Bodansky,[6] with the modification that serum inorganic phosphate was determined in a mixture containing serum, buffer, and substrate, in every way identical with the hydrolysis mixture except that trichloracetic acid was added immediately after addition of the substrate. Because of the interference of both the substrate (sodium beta glycerophosphate) and the trichloracetic acid with the development of color in the Kuttner-Lichtenstein method for inorganic phosphate (Bodansky[7]), the values for serum inorganic phosphate are low to the extent of about 10 per cent. Since the inorganic phosphate liberated by hydrolysis is obtained by the difference between this first determination and one after two hours' incubation at constant temperature, the unit values for phosphatase activity are reasonably accurate without correction. For the phosphate analyses we used a photoelectric colorimeter with a suitable spectral filter. Since the instrument is caliberated with phosphate standards

over a wide range of concentrations, deviations from the Beer's law do not introduce an error.

There was no possibility of maintaining either the experimental or normal subjects on a standard diet, and dietary variations may possibly have contributed to the variability of phosphatase activity observed in individuals from month to month, since Freeman and Farmer[8] demonstrated that values may increase on a high carbohydrate diet and decrease as a result of a high protein diet. Venous blood samples were taken routinely in the afternoon shortly after lunch. Bodansky and Jaffe[9] indicated that there is no detectable difference between samples taken immediately after a meal and after a brief fast.

In Table I are given the phosphatase values for a control group of 19 adult males. With one exception (a man of 49, average blood pressure 210/120) all were between the ages of 17 and 28 and gave normal average blood pressure readings, which facts are partial evidence of good general health.

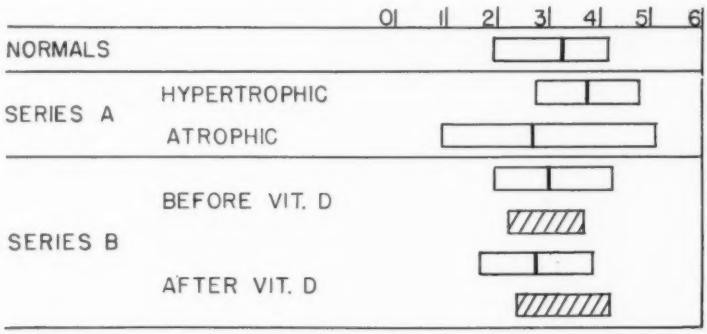


Fig. 1.—Distribution of the phosphatase activity of all experiments. Scale represents Bodansky units; heavy lines dividing rectangles, averages; cross hatched rectangles, series B, serum inorganic phosphate. Scale represents mg. phosphorus per 100 c.c. of serum.

The highest phosphatase activity encountered was 4.41 units, and the lowest 1.90. In 6 of the 19 subjects, or 32 per cent, monthly variations exceeded 1 unit, the greatest variation being 1.66 units. As shown in the last column, the averages of the three or four determinations on a single subject ranged from 1.94 units to 4.12 units, with a general average of 3.21. All of these fall within, or reasonably close to, the normal range of 1.5 to 4.0 units, as defined by Cantarow.[10]

In contrast with these results, observe the spread of values in Table II, in which are summarized the values for group A, arthritic patients receiving vitamin D. The lowest value encountered was 0.44 units, patient E. B., and the highest 5.40, patient F. J. The individual averages revealed a spread of values from 0.92 to 5.08 units. The general average for the 18 atrophic patients was 2.69 units, and for the 5 hypertrophic patients, 3.67. In 14 of the 23 atrophic and hypertrophic patients (61 per cent) the variations from month to month exceeded 1 unit, the greatest being 2.65 units in patient B. B.

These group averages and the spread of values are graphically presented in Fig. 1, which also includes the comparable data for the normal control subjects.

TABLE II

SERUM PHOSPHATASE ACTIVITY, BODANSKY UNITS, AFTER PROLONGED ADMINISTRATION OF VITAMIN D. ATROPHIC AND HYPERTROPHIC ARTHRITIS

SUBJECT	AGE WT.	SEX	FIRST DET.	FIRST MO.	SECOND MO.	THIRD MO.	FOURTH MO.	FIFTH MO.	SIXTH MO.	SEVENTH MO.	NINTH MO.	AVERAGE PHOSPHATASE	VARIATION GREATER THAN 1 UNIT	TREND PHOSPHATASE
Atrophic Series														
E. M.	36/140	F	2.03	2.40	2.10	2.17	2.21		1.87	2.18		2.14		Little change
E. C.	30/130	F	2.28	2.66		1.80	1.58	1.67	2.21			2.03	1.08	Random
V. C.	20/90	F	2.40	2.76	2.62					2.22		2.50		Little change
N. S.	29/149	F	2.99	3.39	3.57	3.64		2.49	3.16			3.21	1.15	Random
E. B.	34/120	F	1.18	1.36		0.68			0.44			0.92		Downward
G. T.	49/130	F	4.29	3.62		2.67	3.67					3.56	1.62	Random
L. K.		F	2.36	2.13	1.05	2.13						1.92	1.31	Random
P.	26/126	F	1.98	1.67	1.71							1.79		Little change
B. B.	59/159	F	3.72		1.99	2.49		4.64				3.21	2.65	Random
A. L.	26/127	M	3.04	2.93	1.36	2.46	2.99					2.56	1.68	Random
W. M.	52/165	M	3.72	2.72	2.75	2.21	2.58	2.75				2.79	1.52	Random

TABLE II—CONT'D

W. G.	43/169	M	2.33	3.29	3.51	2.68	3.29		2.75			2.97	1.18	Random
F. J.	43/194	M	4.57	5.40	5.25	5.31			4.78	5.18		5.08		Little change
H. S.	59/180	M	2.77		2.42				2.50			2.56		Little change
M. P.	61/134	F	2.40		3.12	2.76	2.51	2.36			3.37	2.75	1.01	Random
M. H.	62/156	F	4.07		2.99							3.53	1.08	Random
J. N.	52/110	F	2.95	3.47	3.08	3.26	2.43	2.16	1.95	3.18		2.81	1.52	Random
A. H.	39/147	F	2.75	1.92	1.83	1.96	2.36			1.58		2.07	1.17	Random
											Average	2.69		
Hypertrophic Series														
T. V.	58/115	F	2.81	2.78	2.65							2.75		Little change
O. J.	49/173	M	3.24			2.82	3.06	3.12		2.37		2.92		Random
R. T.	66/130	F	3.38		3.08	2.90	3.88	3.29		3.36		3.32		Little change
K. H.	49/148	F	5.28	4.78		4.30	4.85		4.20	4.10		4.59	1.18	Downward
A. P.	61/176	F	5.37	5.28	5.10	4.91	4.19	3.80				4.76	1.48	Downward
											Average	3.67		

There is no apparent correlation between the phosphatase activity and the clinical picture in this group of patients, as indicated by the following brief protocols, representing patients who received varying degrees of relief as the result of vitamin D therapy.

Patient: E. M.

Date of Admission: July, 1936.

Complaining of: Pain, stiffness, swelling and deformity of hands, left knee, ankles, and feet (seven years' duration).

Findings to Date: March 3, 1938. The swelling was much decreased; pain was present only when grasping objects. She had no pain in hands when washing dishes, although the stiffness was about the same, and she was still unable to close her fingers tightly. The flexion of the left knee was markedly improved; she was able to extend knee fully, and there was very little swelling. There was no pain in knee on resting or walking. Very little swelling in the left ankle, although the right ankle was still swollen, and both ankles gave pain when patient walked or stood for any length of time. There was no improvement in the ankles as far as pain was concerned, and both feet were still painful.

Summary.—There was improvement in the hands and the left knee, but no improvement in the ankles and feet. First phosphatase determination was made on Feb. 2, 1937. Serum phosphatase showed fair constancy, slightly above 2 units.

Treatment: The patient received 150,000 units of vitamin D (ertron) daily, with period of therapeutic vacation.

Patient: E. C.

Diagnosis: Atrophic arthritis.

Date of Admission: October 1, 1937.

Complaining of: (1) Pain, swelling, stiffness and limited motion of both elbows; (2) pain, swelling, stiffness and limited motion of both wrists; (3) pain, swelling, stiffness and limited motion of hands and fingers; unable to close fingers and had a very weak grip; (4) pain, swelling, stiffness and flexion deformity in both knees; (5) pain, swelling, stiffness and limited motion in both ankles; (6) pain and swelling in neck and jaw (four years' duration).

Findings to Date: March 17, 1938: When patient was first admitted, she complained of severe pain in both elbows on pressure, and was unable to move elbows on account of pain. She had no pain at this observation in either elbow on ordinary motion, and only slight pain on pressure. She was able to comb her hair, and could lift a pail of water with no pain whatsoever in either elbow. The pain, swelling, and stiffness in both wrists were much decreased, and there was a slight increase in motion of wrists; she also had greater power in both wrists. She could now wring out towels with no discomfort, while on admittance she was unable to perform these tasks. The swelling and flexion deformity of hands and fingers were greatly decreased and she was able to close fingers completely, with a very good grip, whereas on admission she could not use a pencil to write on account of this severe pain in fingers. The pain, swelling, and stiffness of both knees were much less; she was able to walk more freely and to straighten out knees more readily. She walked 8 blocks to and from work without any discomfort; she was unable to do this when admitted. She had greater mobility in ankles; swelling and pain were much decreased, with only occasional pain in ankles when walking.

Summary: On admittance to the clinic this patient was tired and weak, the slightest exertion exhausted her. She had gained about 20 pounds and was stronger and did not tire so easily. She was unable to hold a job on admittance, but had been employed since Jan. 5, 1938, and worked eight hours a day, made her own meal on coming home, and was able to do this without any strain. On admittance she had pain all the time whether resting or in mo-

tion. She had pain only occasionally on walking at the final observation. First phosphatase determination was made on Feb. 2, 1937. Serum phosphatase varied at random with tendency toward a decrease over the total period of observation.

Treatment: The patient received 180,000 units of vitamin D (viosterol); treatment continued.

Patient: A. L.

Diagnosis: Atrophic arthritis.

Date of Admission: Jan. 14, 1937.

Complaining of: Pain, swelling, and stiffness in all joints (two months' duration).

Findings to Date: April, 1938. On admission patient could not dress or feed himself on account of severe pain, swelling, and stiffness in both shoulders, elbows, wrists, hands, and fingers. He came to the clinic in a wheelchair; he could not walk on account of the pain in the ankles and knees.

Summary: At final observation this patient was working eight hours a day, was able to dress and feed himself, could walk without pain or any discomfort. First phosphatase determination was made on Jan. 14, 1937. A decline of phosphatase activity in early months of vitamin D therapy was followed by a rise, similar to patients of series B, Table III.

Treatment: 150,000 units of vitamin D (ertron); discontinued in October, 1937.

Patient: W. G.

Diagnosis: Atrophic arthritis.

Age: Thirty-five years.

Date of Admission: November, 1936.

Complaining of: Flexion deformity of the right elbow; pain, swelling, and stiffness of both wrists, both hands, and all fingers; flexion deformity of left knee and right ankle. Gradual onset (four years' duration).

Findings to Date: April 13, 1938. On admission the patient could not dress or feed himself, both hands were weak and painful. He could not lift light objects. At present he can do all of these without difficulty. The stiffness and swelling of both hands were improved. The swelling, stiffness, and pain in the left knee were completely absent, and he could walk long distances without any pain, whereas on admission to the clinic he could walk only two blocks and then had to rest on account of pain in left knee and right ankle. The flexion deformity had improved to the extent that he could extend the knee to about 165°.

Summary: The patient was much stronger; he could walk with less difficulty. His wrists, hands, and fingers were stronger, although the stiffness and swelling were still present; pain was absent, except on exertion. The patient had gained 18 pounds. First phosphatase determination was made on Jan. 28, 1937. Phosphatase varied at random over a range of 1.18 units.

Treatment: This patient received 150,000 to 200,000 units of vitamin D daily; he is still under treatment.

Patient: F. J.

Date of Admission: July, 1936.

Complaining of: The patient complained of pain in the back of neck, pain on motion of both shoulders, pain and flexion deformity of both elbows, with "olecranon bursitis" in both elbows. He also complained of pain, stiffness, swelling and limited motion of hands and fingers, pain and stiffness of both knees, pain, stiffness, and swelling of both ankles, and severe pain on the soles and heels of both feet (three years' duration).

Findings to Date: March 3, 1938. The pain in the back of neck had completely disappeared. There was only slight pain in the shoulders, and he could move shoulder joints without feeling any pain. He had no pain in the elbows; however, the deformity had not improved. The motion was still limited, but he could move elbows without any pain. The

swelling in the hands varied. He was able to use his hands without pain, whereas on admission he could not hold a cup in his hand. He now had a good grip and could work, i.e., hold a hammer firmly, etc. He was able to close fingers almost fully at times. He had no pain in knees, only slight pain on arising in the morning, which disappeared within fifteen minutes. He had no more pain in either ankle, although the swelling was still prominent. The heels and soles of both feet were painless, and he was able to walk long distances without pain or discomfort.

Summary: This patient was badly crippled and unable to walk when he was admitted to the clinic. In fact, at times he had to miss visits to the clinic because he was not able to walk to the street car. He could not feed himself because of severe pain in hands and fingers. At final observation he could do manual work without discomfort, and he could walk long distances without any pain. He claimed he felt much stronger generally. First phosphatase determination was made on Feb. 4, 1937; phosphatase was very high. His condition remained quite stable around 5 units.

Treatment: The patient received 150,000 to 250,000 units of vitamin D (ertron) daily and is still under treatment at 200,000 units per day.

TABLE III

SERUM PHOSPHATASE ACTIVITY IN ATROPHIC ARTHRITIS. VITAMIN D ADMINISTRATION BEGUN AFTER SECOND PHOSPHATASE DETERMINATION

SUBJECT	SEX	FIRST DET.	MONTHS AFTER FIRST DETERMINATION				AVERAGE PHOSPHATASE BEFORE	AVERAGE PHOSPHATASE AFTER
			SECOND	THIRD	FOURTH	FIFTH		
T. U	F	2.23 2.12* 1.66	1.39		1.88		2.18	1.64
A. M.	F	3.96 4.50* 3.53	3.18	4.05			4.23	3.59
E.	F	3.23 2.22* 3.29	2.71		2.03	1.50	2.83	2.38
J. M.	F	2.09 1.83* 1.99	1.37	1.58	2.18	1.62	1.96	1.75
E. G.	F	3.56 3.49* 4.65	3.00	3.58 4.24			3.53	3.82
R.	F	2.82 3.42* 1.88	2.94 3.07 5.20 2.95	2.41 3.66			3.12	3.10
					Group average		2.98	2.71

*Beginning of vitamin D administration.

Table III summarizes the results of frequent phosphatase activity determinations on the 6 atrophic patients of group B. The results show that the phosphatase activity has been depressed by the vitamin. This is demonstrated graphically in Fig. 1; along with the uncorrected serum inorganic phosphate values, which show a reciprocal shift, there is a tendency for the phosphatase activity to fall sharply immediately after the beginning of medication, with a tendency toward a later rise. A few typical protocols are included to show

that improvement in the clinical picture seems to correspond with the magnitude of the difference between premediation and postmedication averages. The group is small, however, and we do not feel it safe to press this point.

Patient: T. U.

Diagnosis: Atrophic arthritis.

Date of Admission: March 11, 1937.

Complaining of: (1) Pain, stiffness, and flexion deformity of both elbows; (2) pain, swelling, stiffness, and limited motion of both wrists; (3) both hands and all fingers painful and swollen; (4) pain, swelling, and stiffness in both knees; (5) pain, swelling, and stiffness in both ankles (three years' duration).

Findings to Date: March 17, 1938. There was less stiffness and flexion deformity in elbows and the patient could extend both elbows more easily than on admission. She had very little pain in either elbow, and she could use her wrists more freely. The swelling on the right wrist had decreased, but she had a huge ganglion over the left wrist. She complained of no pain in hands or fingers, and could close fingers, whereas on admission she could not do this due to the severe pain. She had a good strong grip. The swelling in both knees had decreased. On admission she had severe pain in knees either on resting or walking. She was now able to walk 1 mile or more without pain in either knee. The pain and swelling in both ankles were definitely decreased, and there was no pain in ankles when walking.

Summary: The general condition of this patient had improved greatly. She was weak and the slightest exertion exhausted her when she first came to the clinic. She gained about 5 pounds. She was not able to walk when first she came to the clinic, but she was now able to do light housework with no discomfort.

Treatment: This patient received 150,000 to 200,000 units of vitamin D (ertron) since admittance. She is still under treatment.

Patient: A. M.

Date of Admission: April, 1937.

Complaining of: Pain, swelling, and crepitation of both knees.

Comparative Findings: April 14, 1938. On admission the patient had severe and continuous pain in both knees. She was unable to sleep and she could not walk on account of pain. At the latter date she was able to walk 8 or 9 blocks with very slight pain. Crepitation was still present in knees, but no swelling.

Summary: On admission this patient could hardly walk. A year later she could walk with very little difficulty. Crepitation was still present, but there was no swelling.

Treatment: This patient received 150,000 units vitamin D (ertron) with no periods of vacation. She is still under treatment.

Patient: J. M.

Age: Fifty-three years.

Diagnosis: Atrophic arthritis.

Date of Admission: April 15, 1937.

Complaining of: (1) Pain and flexion deformity of both elbows; (2) pain, swelling, and limited motion of both wrists and both hands; (3) pain, swelling, and stiffness of all fingers; (4) pain and swelling in both knees; (5) pain in both feet and all toes (eight years' duration).

Findings to Date: March 10, 1938. The patient still complained of pain and flexion deformity in both elbows, and pain and swelling in both wrists; also of pain and slight swelling in her hands and fingers; less pain in knees, feet, and toes. She was able to walk more freely.

Summary: There was no improvement in the elbows, wrists, and hands, but there is less pain in knees, feet, and toes.

Treatment: The patient received 150,000 to 250,000 units of vitamin D (ertron) daily with periods of therapeutic vacation. She is under treatment.

Patient: P. R.

Diagnosis: Atrophic arthritis.

Date of Admission: May, 1936.

Complaining of: Pain in shoulders, elbows, wrists, fingers, knees, and ankles (two years' duration).

Comparative Findings: July 21, 1938. Patient still complained of severe pain in all joints.

Summary: Condition showed no improvement.

Treatment: This patient received 150,000 to 400,000 units of vitamin D and is still under treatment at the clinic.

DISCUSSION AND SUMMARY

Those patients who were examined for serum phosphatase activity after prolonged medication with vitamin D have, in general, a lower serum phosphatase activity than do healthy normal subjects. That this is an effect of the vitamin in large doses there is little doubt. Page and Reside[11] observed lowered phosphatase activity after large doses of vitamin D, attributing the fall to toxic impurities in the preparation. Crimm and Strayer,[12] however, believed that the lowered phosphatase value is a manifestation of the toxicity of high dosage with vitamin D, independent of impurities. There is no evidence that the arthritic state in itself is associated with a lowering of phosphatase activity; indeed, in those cases investigated before medication both high and low values were encountered, and in the large series summarized in Table II, there were many values above normal, especially in the hypertrophic cases, despite prolonged vitamin D administration. The serial determinations on the individuals in this study who had been receiving vitamin D over a long period of time show either a random variation about the average value or a downward trend. The tendency for the values to rise after an initial fall in the B group of patients might be ascribed to a developing tolerance to the toxic effects of the drug, after which a plateau is reached, this being the approximate status of the A group.

The fact that no apparent correlation exists between the absolute value of the phosphatase activity in units and the degree of clinical improvement in the A series (Table II) is perhaps to be expected, since Table III (Group B) indicates the possibility of a correlation between degrees of improvement and magnitude of the shift from premedication values, but not between improvement and absolute phosphatase values.

In this regard it should be profitable to study the phosphatase activity in arthritis under other types of treatment to ascertain whether the improvement obtained in a high percentage of cases as the result of vitamin D therapy is a specific response to the reduction in phosphatase activity which vitamin D causes in susceptible patients, or whether improvement and reduction in phosphatase activity are merely coincidental.

CONCLUSIONS

It is concluded from this study, first, that serum phosphatase activity does not promise to be of value as an objective diagnostic aid in arthritis due to the wide distribution of values above, below, and within the normal range. The possibility of its value as an aid in differentiating between the atrophic and hypertrophic types is again negated by the wide distribution, regardless of the somewhat higher average obtained by us on a limited number of hypertrophic cases.

Second, there is a possibility that the shift in the serum phosphatase activity, which we have observed to occur in patients who have ultimately benefited from vitamin D, might prove to constitute a basis for prediction as to the suitability of such therapy. Further investigation is needed on this point.

REFERENCES

1. Kay, H. D.: Phosphatase in Growth and Disease of Bone, Physiol. Rev. **12**: 384, 1932.
2. Morris, N., and Peden, O. D.: Plasma Phosphatase in Disease. A Review, Quart. J. Med. **6**: 211, 1937.
3. Kay, H. D.: Plasma Phosphatase. II. The Enzyme in Disease, Particularly in Bone Disease, J. Biol. Chem. **89**: 249, 1930.
4. Race, J.: The Phosphatase Test in the Arthritides and Osteitides, Arch. M. Hydrol. **10**: 6, 1932.
5. Dreyer, I., and Reed, C. I.: Treatment of Arthritis With Massive Doses of Vitamin D, Arch. Phys. Therapy **16**: 537, 1935.
6. Bodansky, A.: Determination of Plasma Phosphatase, Proc. Soc. Exper. Biol. & Med. **28**: 760, 1931.
7. Bodansky, A., Hallman, L., and Bonoff, R.: Phosphatase Studies. I. Determination of Inorganic Phosphate, Beer's Law and Interfering Substances in the Kuttner-Lichtenstein Method, J. Biol. Chem. **99**: 197, 1932-1933.
8. Freeman, S., and Farmer, C. J.: Correlated Studies of Calcium, Inorganic Phosphorus, and Serum Phosphatase in Animals Influenced by Ergosterol, Am. J. Physiol. **113**: 209, 1935.
9. Bodansky, A., and Jaffe, H. L.: Phosphatase Studies. III. Serum Phosphatase in Diseases of the Bone. Interpretation and Significance, Arch. Int. Med. **54**: 88, 1934.
10. Cantarow, A.: Review of Phosphatase Activity, and Calcium Electrolyte Metabolism, Internat. Clin. **1**: 230, 1936.
11. Page, I. H., and Reside, D. M.: Die Wirkung von bestrahlten Ergosterin auf Gewebe-Phosphatase, Biochem. Ztschr. **223**: 171, 1930.
12. Crimm, P. D., and Strayer, J. W.: Phosphatase Content of Blood Serum and Tissues in the Rat Following Administration of Vitamins D and A, J. Biol. Chem. **112**: 511, 1936.

THE NEURAL DEPRESSING EFFECT OF TRICHLORETHYLENE*

H. S. Rubinstein, Ph.D., M.D., E. Painter, Ph.D., and O. G. Harne
Baltimore, Md.

TRICHLORETHYLENE has been used with a fair degree of success in the treatment of trigeminal neuralgia (Plessner, 1916; Glaser, 1931). The beneficial effect derived in these cases was considered to be due to the specific depressant action which this chemical exerted upon the trigeminal nerve. For this reason, and because of the preponderant innervation of the dura by the trigeminal nerve (Foerster, 1926), this same drug was used in a series of migrainous patients with encouraging results (Rubinstein, 1937).

However, because examination of such treated patients failed to disclose a limited trigeminal anesthesia which observation substantiated the findings of others (Goldberg, 1924; Bieder, 1921), it became questionable whether trichlorethylene did produce its effect through a limited specific action. The following study was therefore undertaken.

A series of dogs was anesthetized with ether and the dura mater and right sciatic nerve of each were exposed. Each animal was then allowed to come to a very light anesthetic stage. Observations were then made in which the dura mater and sciatic nerve were stimulated with a faradic current. Since the dura is free from nerve endings in many of its areas, exploratory stimulation was carried out until a response was obtained (see Fig. 1). This selected point was then used for subsequent stimulation. The response was measured as deviations in blood pressure obtained through a recording manometer inserted into the carotid artery.

Following a series of responses observed under deep and light ether anesthesia, the animal was allowed to inhale 2 to 3 c.c. of trichlorethylene administered through a tracheal cannula. The animal soon went into a deep sleep during which time stimulation of the dura or sciatic failed to produce any reflex effect upon blood pressure. As sleep wore off the dura and sciatic nerves were again stimulated with faradic current and observations were again made. Fifty-two such observations were made before and 52 after the use of trichlorethylene.

All data obtained were tabulated and treated by the usual statistical methods (Pearl, 1930). Means for each series of observations were then compared with their respective controls and ratios of significance were determined. Ratios of 3 or more were considered to be significant.

*From the Research Laboratory, Surgical Division, Sinai Hospital, Baltimore; Department of Physiology, College of Physicians and Surgeons, Columbia University, New York; and the Department of Physiology, School of Medicine, University of Maryland, Baltimore.

Received for publication, January 12, 1939.

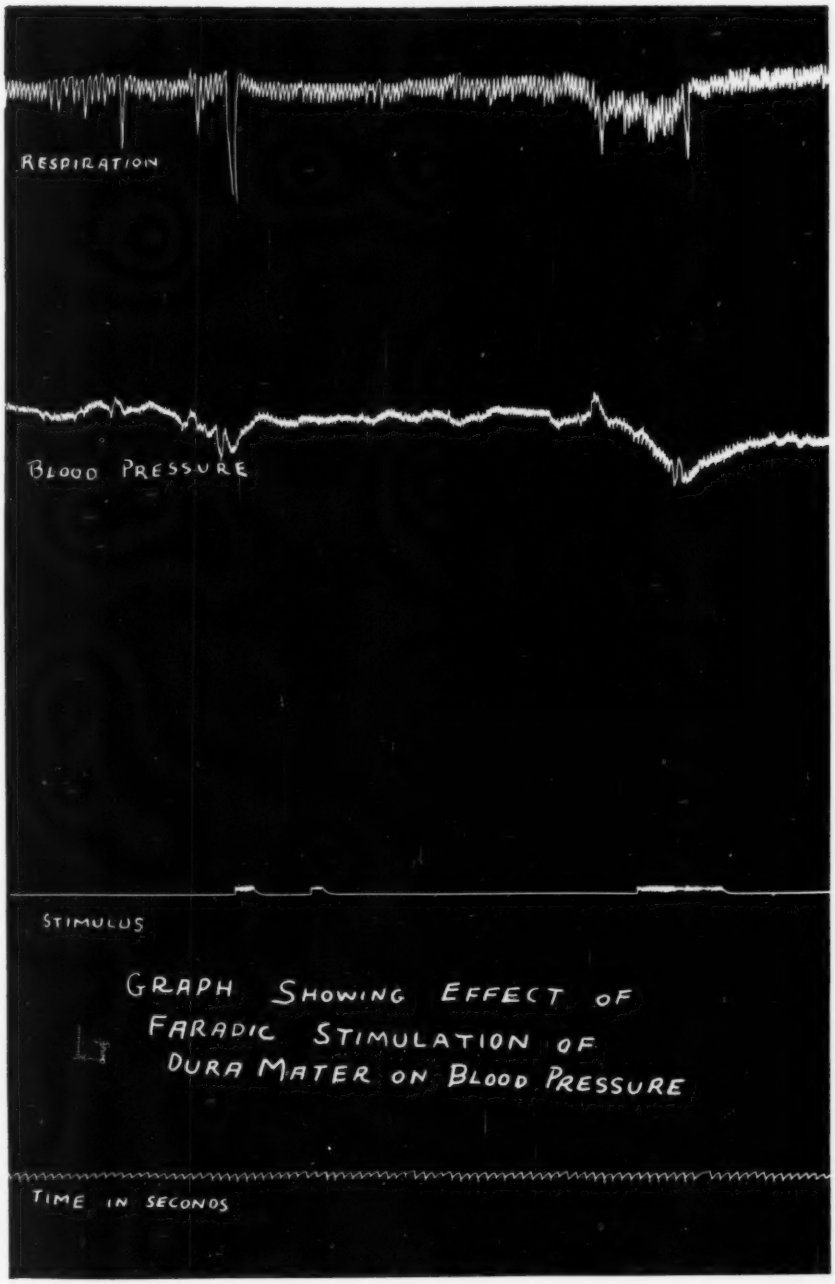


Fig. 1.

RESULTS

From Tables I and II it will be noted that before the inhalation of trichlorethylene, i.e., when the animal was merely under very light ether anesthesia, sciatic and dural stimulation led to significant deviations in blood pressure.

As long as the animal was in deep sleep, after the administration of trichlorethylene, no response was obtained following stimulation. In this respect it resembled the lack of response during deep ether anesthesia.

TABLE I

COMPARISON OF BLOOD PRESSURES UNDER LIGHT ETHER ANESTHESIA BEFORE THE USE OF TRICHLORETHYLENE

BEFORE SCIATIC STIMULATION	AFTER SCIATIC STIMULATION	DIFFERENCE	CRITICAL RATIO
Blood pressure 111 ± 1	103 ± 2	8 ± 2.2	3.62
BEFORE DURAL STIMULATION	AFTER DURAL STIMULATION		
Blood pressure 111 ± 1	101 ± 1	10 ± 1.4	7.14

TABLE II

COMPARISON OF BLOOD PRESSURES UNDER LIGHT ETHER ANESTHESIA AFTER THE USE OF TRICHLORETHYLENE

BEFORE SCIATIC STIMULATION	AFTER SCIATIC STIMULATION	DIFFERENCE	CRITICAL RATIO
Blood pressure 111 ± 0.8	104.5 ± 3.2	6.5 ± 3.3	2
BEFORE DURAL STIMULATION	AFTER DURAL STIMULATION		
Blood pressure 111 ± 0.8	108 ± 1.0	3 ± 1.2	2.5

As the animal began to awaken (following treatment with trichlorethylene) early stimulation of both dura and sciatic nerves failed to produce significant change in blood pressure (Table II). As the effects of trichlorethylene wore off, a stage was reached where sciatic stimulation resulted in a reflex response. Immediately thereafter stimulation of the dura likewise resulted in such a change. In other words, when trichlorethylene was sufficient to inhibit the dural-medullary reflex, it was also sufficient to inhibit the sciatic-medullary response. When recovery was complete enough to permit the sciatic-medullary reflex to function, almost immediately a similar response was obtained from dural stimulation.

DISCUSSION

In deep ether anesthesia it is well known that all reflex pathways are depressed in their conductive power. For this reason ether is now regarded as a general anesthetic. As ether anesthesia becomes lighter, reflex pathways begin to respond—again in a general fashion. If trichlorethylene were at all specific for the trigeminal sensory mechanism, one would expect that at some time during the course of recovery from effects produced by its inhalation, reflexes dependent upon trigeminal sensory integrity would be abolished.

From these experiments it was noted that when (as a result of trichlorethylene administration) the animal was asleep, all sensory reflexes were abolished.

Similarly, as long as the animal was sufficiently under the influence of the drug to prevent a successful dural response, the sciatic response was also unobtainable. However, when recovery from the anesthetic action of the drug was sufficient to lead to sciatic response, dural stimulation likewise resulted in blood pressure change. From this it is seen that trichlorethylene, like ether,

acts as a general anesthetic, obtunding all sensory elements simultaneously. Unlike ether, however, it is rapid in its effect and allows for quick recovery. Experience on man indicates it is not nauseating.

CONCLUSIONS

Trichlorethylene produces its effect through a general anesthetizing action so that when inhaled in sufficient concentration to depress trigeminal pathways it simultaneously depresses the sciatic sensory system. At no stage of its anesthesia could there be detected a response in one mechanism when a response in the other was not also obtained. Its advantage clinically arises from its quick action, short-lived effect as an anesthetic, and its nausea-free character.

The authors acknowledge the kind cooperation of Dr. David Bryce, of the Calco Chemical Co., for supplying the trichlorethylene used in this study.

REFERENCES

1. Plessner, W.: Über trigeminuserkrankung infolge von trichlorathylenvergiftung, Klin. Wchnschr. **53**: 25, 1916.
2. Glaser, M. A.: Modern Methods for the Relief of Tic Douloureux, West. J. Surg. **39**: 901, 1931.
3. Foerster, O.: Die Leitungsbahnen des Schmerzgefühls und die chirurgische Behandlung den Schemrzzustände, Berlin, 1926, Urban und Schwarzenberg.
4. Rubinstein, H. S.: The Use of Trichlorethylene in the Treatment of Migraine, Arch. Neurol. & Psychiat. **37**: 638, 1937.
5. Goldberg, E.: The Effect of Trichlorethylene (Chlorylene) and the Indications for Its Therapeutic Use, Deutsche Ztschr. f. Nervenh. **82**: 10, 1924.
6. Bieder: The Problem of Trichlorethylene Treatment of Trigeminal Neuralgia, Inaug. Diss., Breslau, 1921.
7. Pearl, R.: Medical Biometry and Statistics, Philadelphia, 1930, W. B. Saunders Co

---

THE MECHANISM OF GOLD THERAPY IN RHEUMATOID ARTHRITIS*

DAVID H. KLING, M.D., LOS ANGELES, CALIF., AND DAVID SASHIN, M.D., AND JOSEPH SPANBOCK, M.D., NEW YORK, N. Y.

THE efficiency of the treatment of rheumatoid arthritis by the injection of various inorganic, organic, or colloidal gold solutions has been established by a number of European authorities for over a decade. It is gaining recognition and importance recently in the United States. Elsewhere[1] we have given an analysis of the results of nearly 1,600 cases of rheumatoid arthritis selected from the literature and 80 cases of our own observation. We have come to the conclusion that this therapy produces a high percentage of marked improvement even in advanced cases of rheumatoid arthritis. After repeated courses of treatment relapses are rare, and it is the consensus of opinion that gold therapy yields far more benefit in rheumatoid arthritis than any other measure. On the other hand, toxic reactions occur frequently and are sometimes serious. The frequency and severity of toxic reactions depend primarily on the dosage. The localization of the toxic reaction is influenced also by

*From the Arthritis Clinic, White Memorial Hospital, Los Angeles, and the Arthritis Clinic and Orthopedic Service of Dr. H. Finkelstein, Hospital for Joint Diseases, New York.
Received for publication, February 1, 1939.

the type of preparation used. For instance, sanocrysin (a double thiosulfate of sodium and gold) has a higher toxicity on the kidneys, while solganol B (gold thioglucose) affects especially the gastrointestinal tract. Myocrisine (aurothiomalate of sodium) produced in Hartfall, Garland, and Goldie's[2] series a higher percentage of jaundice than other preparations.

The therapeutic, as well as the toxic, effects of gold preparations indicate the importance of an elucidation of the distribution and action of gold salts in the body. In this paper the facts which have been ascertained are reviewed and new observations are reported which may form the basis for a more adequate explanation of the mechanism of gold therapy in rheumatoid arthritis. Our knowledge in this respect is yet incomplete.

Bactericidal Effect of Gold Compounds.—Gold belongs to the group of heavy metals and shares with them the property of inhibiting bacterial growth in organic material, the so-called oligodynamic effect. Feldt[3] found that gelatin media containing one part of colloidal gold in 6,000 parts of gelatin remain sterile although left open for forty-five days. In control tubes a heavy growth developed within three days. In aqueous solutions gold cyanide inhibits the growth of tubercle bacilli in a dilution of 1:2,000,000 (Koch,[4] DeWitt[5]) and colloidal gold in a dilution of 1:1,000,000 (Feldt). According to Behring, the inhibitory power of gold was reduced in blood serum to only 1:20,000 or 1:30,000. The bactericidal effect of gold compounds on the tubercle bacillus was found to be slight (Koch). DeWitt and Sherman[6] reported that a solution of 1:1,000 of gold chloride is required to kill the tubercle bacillus. Gold tricyanate was not constantly able to kill all tubercle bacilli even in a concentration of 1:100. Feldt came to the conclusion that in vitro gold compounds have a very slight bactericidal effect on recurrent spirochetes or nagana trypanosomes. DeWitt treated a series of guinea pigs infected with tuberculosis by subcutaneous injections of a compound of gold cyanide with cantharidin (aurocantan). This compound inhibited growth of the tubercle bacillus in a concentration of 1:250,000. From 30 to 50 per cent was eliminated in the urine and feces in one week. Therefore, the injections of 2 to 10 mg. were spaced in weekly intervals in order to assure a concentration sufficient to prevent the growth of tubercle bacilli. The results were entirely negative. Not only was a therapeutic effect missing, but also the treated animals showed a tendency to hemorrhage and hyperemia, and there was a marked shortening of life compared with the untreated control animals. Feldt previously admitted that in guinea pigs effects of this gold compound are not marked; however, in rabbits there was an increase in weight, prolongation of life, and replacement of tubercles by scar tissue. Feldt later developed the newer organic gold preparations by the combination of gold with a sulfur compound which decreased the toxicity and permitted an increase in the dosages. By intravenous injections of these compounds, sanocrysin, solganol, etc., he was able to destroy syphilis in rabbits with an efficiency equal to that of salvarsan. Recurrent spirochetes were made to disappear in twenty-four hours from the blood of infected mice. He claimed efficiency of these gold compounds also in streptococcic infections of mice which have a high grade of resistance toward chemotherapy. He concluded on the basis of the outlined animal experiments that the therapeutic

effect of gold on experimental animals is not due directly to its bactericidal properties, but to a stimulation of the defense mechanism, especially of the reticulo-endothelial system.

In the therapy of human diseases gold compounds were used extensively since 1891 in visceral and skin tuberculosis. For twenty years heated debates were waged as to the efficiency of this treatment. The high dosages used were followed by severe reactions, and inconclusive results have brought this therapy into more or less disrepute. Sporadic reports of treatment with gold compounds were published at different times by different workers in a variety of diseases, such as syphilis, cancer, typhoid and paratyphoid fever, chronic rheumatism, and various forms of spinal and cerebral sclerosis. Lewy and Freund[7] claimed cure of recurrent fever in human beings and also subacute and chronic streptococcic septicemias. The reports of the efficiency of gold salts in chronic infections induced Forestier[8] in 1928 to introduce this therapy in rheumatoid and other types of infectious arthritis where they have proved their merit, although in other conditions their use has been almost discarded.

The Distribution of Gold Compounds in the Body.—Older and more recent chemical analyses agree that gold is deposited in higher concentrations in the organs which are the chief representatives of the reticulo-endothelial system. DeWitt found the highest concentration in the spleen of guinea pigs treated with aurocantan, namely, an average of 0.09 mg. per gram of spleen. The liver, the kidneys, the lymph glands, and the skin showed about an equal concentration per gram, approximately 0.021 to 0.026 mg., or only one-fourth that found in the spleen. In other parts of the body and in the blood only minimal amounts of gold were found. The recent work of Köppenhofer[9] has brought out the interesting fact that the mode of distribution of gold in the animal body depends on the preparation used and on the state of health. According to Köppenhofer, organic and inorganic gold compounds injected into normal guinea pigs are deposited to a higher degree in the parenchymal cells of the liver, kidney, spleen, and bone marrow. In tuberculous guinea pigs all gold compounds, irrelevant of their chemical composition, are taken up chiefly by the reticulo-endothelial cells and very little by the parenchymal cells. This difference is explained by an allergic condition of the mesenchymal cells in the infected organism with increased activity. On this basis it appears that in the treatment of patients there would not be a pronounced difference in the action of various compounds. However, it may be safer to use colloidal gold preparations if further observations bear out Köppenhofer's findings, although Forestier claims that colloidal gold preparations have no effect. Our clinical experiences with the colloidal gold preparation, aurocein, also appear to contradict his opinion. These observations justify the conclusion that one of the chief actions of gold compounds is the stimulation of the reticulo-endothelial system to increased activity and production of proteolytic ferments with bactericidal and antitoxic properties.

The Action of Gold in the Rheumatoid and Infectious Types of Arthritis.—Besides the systemic effect of gold through the reticulo-endothelial system, evidence can be presented of a local effect on the diseased joint tissues and

especially on the synovial membrane. Experiments by Eichelburger and McClusky[10] indicate that there is a greater retention of gold in the infected animal. They found that the excretion of gold in the urine of tuberculous animals is 10 per cent less than in normal guinea pigs. Köppenhofer has found gold within the epitheloid cells of tubercles. The synovial membranes contain large amounts of reticulo-endothelial cells. Gold, therefore, will be excreted directly into the synovial membrane. In inflammatory joint affections the number of reticulo-endothelial cells is increased, and the storage of gold compounds and the stimulation of the defense action will be greater. This conception is supported by experiments on rabbits which had received intravenous or intra-articular injections of various gold preparations. Michelazzi[11] found gold in the reticulo-endothelial cells of the synovial membranes. The quantity was small in normal synovial membrane. In inflammatory membranes with cellular proliferation much larger deposits of gold were found. This may explain the clinical observation that cases of pronounced and advanced inflammatory lesions may obtain more spectacular results than less severe cases.

In a series of aspirated synovial effusions from knee joints of rheumatoid arthritis under treatment with gold sodium thiosulfate by Kling, the presence of gold was detected spectrographically by Dr. A. Cheney after the administration of even such small amounts as 350 mg. This observation, therefore, supports the animal experiments on the higher concentration of gold salts in diseased synovial membranes and in joint effusions.

Rheumatoid arthritis is a local as well as a systemic disease. The general and local action of gold compounds on the defense mechanisms explains its efficiency against rheumatoid arthritis. It was experimentally established that gold is not efficient in acute infections. Feldt found that injections of gold at the time of inoculation did not prevent the development of tuberculosis in rabbits, but they were successful if administered some time after inoculation. Lewy and Freund observed no influence on acute streptococcic sepsis but very gratifying results in chronic types of septicemia. The course of rheumatoid arthritis is eminently chronic and gold therapy, therefore, appears to be well adapted to its treatment. Gold compounds are slowly excreted. When given at close intervals, a sufficient concentration may be obtained to maintain a prolonged effect. Added to it is the experimentally proved higher tendency of diseased tissue to store gold. These facts may explain the observed long duration of the therapeutic effect of gold compounds in rheumatoid arthritis and the relatively low incidence of relapses, especially after repeated courses of treatment.

SUMMARY AND CONCLUSIONS

1. Studies on the action and distribution of gold salts are indicated because of the increasing importance of gold therapy of rheumatoid arthritis and the frequency of toxic reactions.

2. The bactericidal effect of gold salts is negligible.

3. In infected animals gold compounds are deposited in the highest concentrations in the reticulo-endothelial system, and one of the chief actions of gold is the stimulation of this system to increased activity.

4. In experimental arthritis of animals as well as in rheumatoid arthritis gold salts are deposited in higher concentrations in the proliferated synovial membrane which contains a large number of reticulo-endothelial cells.

5. In a series of synovial effusions from rheumatoid arthritis gold was detected after administration of only 350 mg. of gold sodium thiosulfate (37 per cent gold).

6. On the basis of experimental and clinical evidence in rheumatoid arthritis the efficiency of gold salts is considered to be due to the stimulation of the general reticulo-endothelial system as well as to the effect of the local deposit of gold on the defense mechanism of the synovial membrane.

REFERENCES

1. Sashin, David, Spanbock, Joseph, and Kling, David H.: Gold Therapy in Rheumatoid Arthritis, J. Bone & Joint Surg. **31**: 723, 1939.
2. Hartfall, S. J., Garland, H. G., and Goldie, W.: Gold Treatment of Arthritis, Lancet **2**: 784, 1937.
3. Feldt, Adolph: Chemotherapeutische Versuche mit Gold, Klin. Wchnschr. **5**: 299, 1926; Klin. Wchnschr. **6**: 1137, 1927.
4. Koch, Robert: The Cure of Tuberculosis, Deutsche med. Wchnschr. **16**: 757, 1890.
5. DeWitt, Lydia M.: The Use of Gold Salts in the Treatment of Experimental Tuberculosis in Guinea-pigs, J. Infect. Dis. **23**: 426, 1918.
6. DeWitt, Lydia M., and Sherman, Hope: Tuberculocidal Action of Certain Chemical Disinfectants, J. Infect. Dis. **15**: 245, 1914.
7. Lewy, F. A., and Freund, R.: Die Behandlung des chronischen Infekts mit dem neuen Goldpräparat Solganal, Deutsche med. Wchnschr. **52**: 1857, 1926.
8. Forestier, J.: Rheumatoid Arthritis and Its Treatment by Gold Salts, J. Lab. & Clin. Med. **20**: 827, 1935.
9. Köppenhofer, G. F.: The Deposition and Distribution of Organic and Inorganic Gold, Deutsche med. Wchnschr. **62**: 1011, 1936.
10. Eichelburger, Lillian, and McClusky, Lucille K.: The Effect of Injection of Sanocrysin on Normal and Tuberculous Dogs, Am. Rev. Tuberc. **14**: 53, 1926.
11. Michelazzi, A.: Experimental Study of Gold Treatment and the Presence of Gold in Synovial Articulations, Gior. di clin. med. **15**: 749, 1934.

THE ANTIGENIC PROPERTIES OF STREPTOCOCCI KILLED BY ULTRAVIOLET LIGHT*

Maynard Murray, B.S., M.D., Cincinnati, Ohio

LACK of evidence to support the hypothesis that pyogenic streptococci fail to produce immunity prompted us to search for a different method of vaccine preparation. Since we felt that the ordinary methods of vaccine production (heat, formaldehyde, oxidizing reagents) unduly denature the microorganismal bodies, we tried light.

As bacterial reactions are so specific it might seem logical that in the preparation of vaccines the substance necessary for the antigenic properties might be altered or destroyed. Thus one might be led to suspect that such a change occurs in cases where we have failed to produce a potent vaccine. There is clinical evidence to support the fact that antibodies are formed by organisms from which we can prepare no useful vaccine while the patient is

*From the Laboratory of Physiology, University of Cincinnati.
Received for publication, February 4, 1939.

suffering from the disease. Syphilis is an excellent example of this condition. Therefore, the infecting organism is not of the same chemical composition as organisms used in the vaccine.

The effect of heat in altering the specificity of a protein reaction is easily demonstrated. Thus guinea pigs sensitized to egg albumen are easily shocked, even to death, upon reinjection of the albumen. But if the egg white is first boiled and the coagulum pulverized, it no longer evokes anaphylaxis.

Our vaccine was prepared by subjecting a dried twenty-four-hour culture to ultraviolet light. The organisms were grown on blood agar plates, washed off with a little saline solution, and desiccated to bare dryness. They were then subjected to the light of a quartz mercury-vapor lamp for at least four hours. The death of the organism was checked by its suspension in saline and culture at daily intervals for three days. They were not read as negative until ninety-six hours later. Growths exposed for less than four hours proved still viable, but their growth had been so slowed that it was not apparent until forty-eight or more hours. When the suspensions proved sterile, they were diluted with sterile physiologic salt solution to proper concentration.

In this experiment 30 rabbits were used. Ten rabbits were given increasing doses of this irradiated (streptococcus) vaccine every other day for a month. The initial dose of 1 minim was thus gradually raised to 1 c.c. The suspension contained approximately 3,000,000,000 bacteria per c.c.

Ten more animals were treated in identical fashion with similar suspensions, but of a vaccine killed by heat.

Ten more animals were used as controls.

Two days after the last dose of vaccine all 30 animals were given 1 c.c. of a suspension of the live organism intravenously.

The 10 untreated animals all died in twenty-four hours.

Of the animals treated with the heat-killed vaccine, 7 died in twenty-four hours; the rest within ninety-six hours. Necropsy showed a frank pneumonia in all.

The 10 animals treated with the irradiated vaccine all lived, only one seeming to have pneumonia.

An interesting observation was made in that the heat-killed vaccine produced a severe skin reaction similar to a severe positive tuberculin reaction twenty-four hours after being injected intradermally. The irradiated vaccine did not. This was contrasted both on goats and human beings.

The evidence, therefore, seemed to be that greater immunity to infection was assured by injection of a vaccine prepared by irradiation than by one heat killed. At the same time the former appeared less toxic than the latter for intradermal test on patients, and experimental animals showed less local redness and swelling.

REFERENCES

1. Gartner, S., and Szothmary, J.: Ueber die Antigenfunktion der mit ultra-violetten Strahlen behandelten Bakterien, Ztschr. f. Immunitätsforsch. u. exper. Therap. 78: 256, 1933.
2. Bechem, A.: Ultra-Violet as a Bactericide, Arch. Phys. Therapy **16**: 733, 1935.

EFFECT OF THYMUS TRANSPLANTS ON TUMOR GROWTH*

MAYNARD MURRAY, B.S., M.D., CINCINNATI, OHIO

IT IS a generally accepted fact that young animals are not as liable to neoplastic growth as older ones. In debating why this may be, it has been suggested that lack of something liberated by a retrogressing embryonic structure, like the thymus, may be responsible.[1] It was on this hypothesis that the following experiments were undertaken.

Our rats were inoculated with a transplantable tumor of the breast, kindly sent us by Francis C. Wood, of the Institute of Cancer Research, Columbia University (Fig. 1 *a* and *b*). The tumor was transplanted under the skin of the abdomen. After two weeks the animals in which a transplant had taken were separated from the others, and after another three weeks they were divided into two groups. At this time the tumors measured 3 cm. in diameter; they caused the skin to become discolored and were almost ready to slough (Fig. 2).

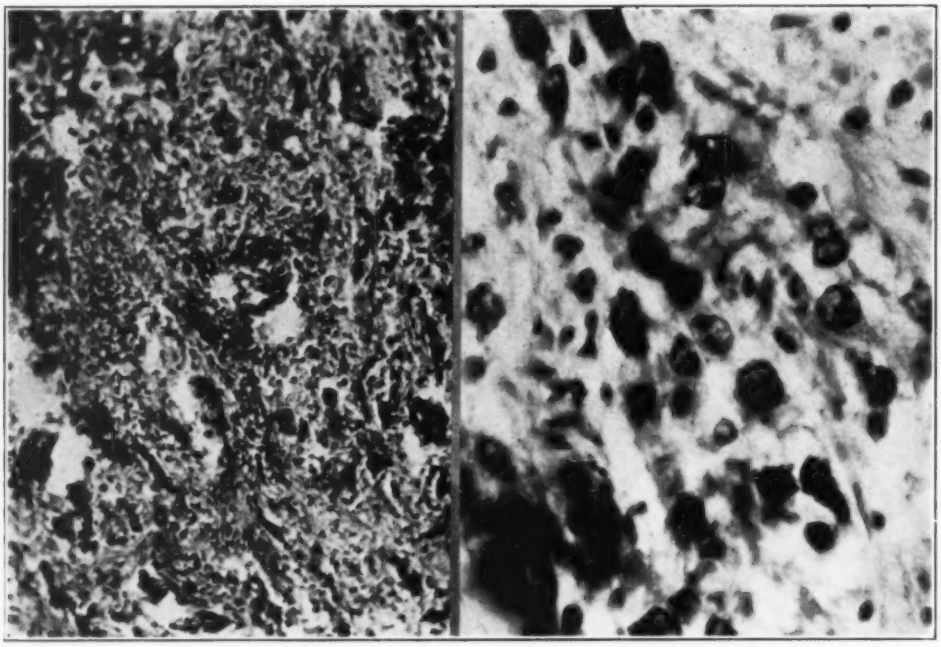
Group A which contained 94 animals was used as control.

Group B containing 87 animals received transplants of fresh thymus taken from rats found immune to tumor transplantation. The thymus glands from 2 such animals were transplanted beneath the skin of each animal in this group. If the transplant was absorbed, another was made one week later. If the gland grew, as it did in 66 of the animals with the first transplant, the tumor became progressively smaller (Fig. 3), so that at the end of four weeks no signs of the neoplasm remained (Fig. 4). In 21 of these animals the first thymic transplant was absorbed, and two more thymus glands were implanted after a week. After the second transplant each of these animals showed retrogressive changes in their tumors identical with those above described, except that 4 of these 21 animals died of pneumonia. All 83 of the animals remaining of this group B seemed perfectly healthy and were bearing young sixteen weeks later.

In group A 3 animals died within the first week after segregation and before their tumors had sloughed. Death was attributed to pneumonia. In the remaining 91 the tumor began to slough one week after segregation, and after another week the animals started to die. At the end of seven weeks all had died except 5, with death attributable to tumor. Direct extension of the tumor to the intestine or metastasis to the lung was usually responsible. Necrosis, toxicosis, and secondary infection caused the death of the others. In the 5 exceptions the tumor had sloughed and apparent spontaneous cure had followed. These animals are seemingly healthy after sixteen weeks.

In a similar series of 60 animals with transplanted tumors five weeks old, 30 were implanted with calf thymus and 30 with the thymus of a six-week-old dog. In each instance 0.5 gm. was implanted, the weight equivalent of 2 rat

*From the Laboratory of Physiology, University of Cincinnati.
Received for publication, February 4, 1939.



a. *b.*

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

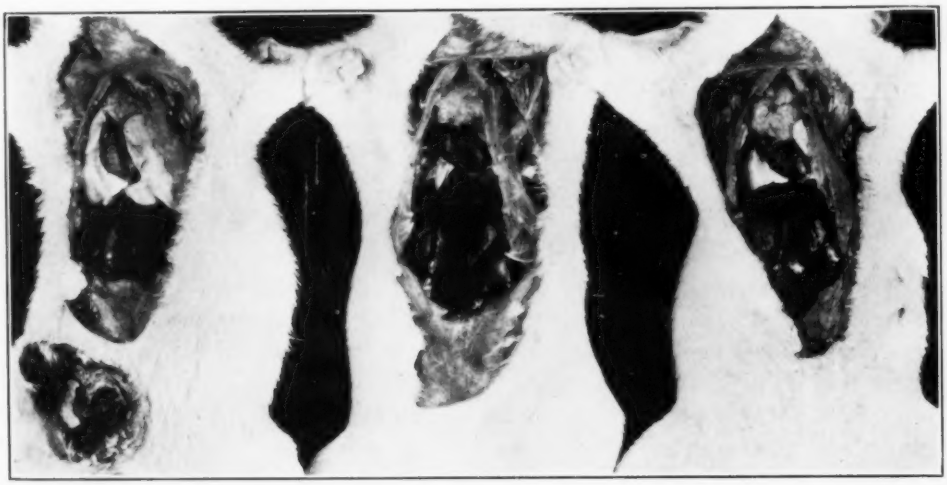


Fig. 5.

thymi. The calf thymus exerted no demonstrable effect upon the tumors, but the dog thymus did. All the tumors became progressively smaller and disappeared as in the animals treated with rat thymus. These animals are alive and well twenty weeks after their experience.

We have noted a direct relationship between the size of thymi in rats and their susceptibility to neoplastic growth. Thus from a 3-animal group of the same litter, 2 were implanted with tumor and one was kept as control. After

five weeks a pronounced difference in the size of the thymus was apparent. The thymus of the animal which did not take the transplant (Fig. 5 *c*) was noticeably larger and the thymus of the animal which took the transplant decidedly smaller (Fig. 5 *a*) than that of the control animal (Fig. 5 *b*). This was proved true in every instance of 25 such series.

CONCLUSIONS

1. Transplanted thymus from rats and dogs exerted marked inhibition of tumor growth and caused them to be absorbed in a high percentage of all rats experimentally inoculated with Francis C. Wood's rat breast tumor. Calf thymus did not show such effect.

2. The thymus was observed uniformly to be extra large in rats found immune to tumor transplants.

REFERENCES

1. Gwyer, F.: On the Thymus Gland Treatment of Cancer, Ann. Surg. **47**: 506, 1908.
2. Thielhaver, A.: Zur Lehre von den Beziehungen des Organismus zum Karzinom, München med. Wchnschr. **74**: 1078, 1927.

INDUCED LOCALIZATION OF STREPTOCOCCI THROUGH CALCIUM GLUCONATE INJECTIONS*

EDWIN G. EBERTZ, B.S., AND WALTER C. LOBITZ, JR., B.S.
CINCINNATI, OHIO

THE phenomenon of selective tissue localization is still subject to dispute. Rosenow[1] has shown how fresh cultures of the streptococci isolated from endocarditis, myositis, cholecystitis, or appendicitis tend to localize on injection into the general blood stream of experimental animals in those regions from which the microorganisms were recovered originally. His findings and the confirmatory evidence of Haden[2] and Nickel[3] emphasize thus the properties of the microorganism as the important factor in such specificity. On the other hand stand the older impressions of clinical observers who maintained that localization was likely to occur in "spots of lowered resistance." Thus they explained, for example, the development of a tuberculosis in a wrenched hip or knee joint, an osteomyelitis after crushing injury, or a skin gumma in a traumatized site.

The following experiments try to bring laboratory proof for the second of these concepts. They show how neither (a) the intravenous injection of a streptococcus, nor (b) the intramuscular injection of calcium gluconate alone produces any pathologic reaction, but how (c) the two together do.

DISCUSSION

Rosenow[4] points out that streptococci lose their specific localizing power when artificially grown. Delitala,[5] inquiring into the possibility of conferring

*From the Department of Physiology, University of Cincinnati.
Received for publication, February 10, 1939.

organotropism upon bacteria through culture in media containing the organ tissues, obtained only negative results. Rhodes and Apfelbach[6] produced an experimentally localizing streptococcal infection after implantation of bone fragments, previously placed in bacterial cultures, in muscle and spleen. Rossides[7] produced abscesses through simultaneous local injection of staphylococci with calcium chloride. Using rabbits and rats, Heubner and Sanders[8] observed that injection of calcium gluconate (apparently without aseptic precautions) caused abscess formation of increasing incidence with increase in quantity and concentration of the salt; the practice of aseptic technique decreased this incidence so that no abscess occurred with 10 per cent solutions. Twenty per cent solution in larger quantity did, however, still cause abscess (on two occasions with necrosis and self-amputation). Goldman[9] observed that therapeutic injections of calcium gluconate intramuscularly in erysipelas often yielded abscesses. While some such abscesses might be attributed to external infection, he felt that others were the consequence of stabilization of an infection carried by the host. Such findings are not reported in clinical observations upon diverse acute infections (Herrold[10]; Oriani, Thoné, and Zau[11]). Von Hofe and Jennings,[12] and Tumpeer and Denenholz[13] report cases of calcium deposition following the intramuscular administration of calcium gluconate in infants. The progress and resolution of these lesions paralleled our experimental work. Chemical analysis by Tumpeer and Denenholz showed the calcium to be calcium phosphate. The patient of von Hofe and Jennings developed inflammatory change over one thigh lesion; softening followed and the pus revealed hemolytic streptococci on culture. They, at that time, placed no emphasis upon the presence of bacteria as of etiologic importance, since their calcified areas did not present the cardinal signs of inflammation. Our experimental lesions, except for slight increase in local temperature, never showed the classic evidences of inflammatory change.

History and review of the subject of calcium deposition is presented by Shands.[14]

We first tried to induce localization of streptococci within a muscle by trauma and a heavy spray of roentgen rays. After failure by these methods we tried to make practical Leon Goldman's observation of the intramuscular injection of calcium gluconate.

METHODS

The streptococci used were cultured and subcultured in a modification of Rosenow's medium[4] from the pulp cavity of an infected tooth removed from a patient the victim of scleredema. Lang and Morris helped us in this matter. The streptococci were nonhemolytic and did not discolor hemoglobin; to reduce their specificity they were cultured in vitro at least one week before use (Rosenow[4]).

The experimental animals were stock albino rabbits injected intravenously with 2 c.c. of a twenty-four-hour subculture (marginal ear vein) at arbitrary intervals, the number and spacing depending upon the reaction of the rabbits to earlier injections. Four cubic centimeters of a 7 per cent solution of calcium gluconate were then aseptically injected into the lateral thigh muscles.

Fig. 1.—Twenty-four hours after the intramuscular injection of calcium gluconate and streptococci intravenously there was a loss of striation, swelling, round-cell reaction, and an increase in cell nuclei in the injected muscle (hematoxylin and eosin stain).

Fig. 2.—The rabbit thigh on the right is normal. The rabbit thigh on the left reveals a rough, yellow white, hard plaque in the anterolateral muscle.

RESULTS

A control group of 6 rabbits given only streptococci showed anorexia, cachexia, and generalized weakness within ten days after the first injection. After death, naturally or by chloroform, necropsy most commonly revealed a suppurative lobular pneumonia. A few showed inflammatory reactions in the endocardium, kidney, or joints.

A second control group was injected intramuscularly with calcium gluconate. Biopsies from the injected areas taken at seven-, fourteen-, twenty-one-, twenty-eight-, and forty-two-day intervals, and at the end of six months, revealed no pathologic change. Cultures made from the hashed excised tissues were negative.

A third control group of 6 rabbits was injected intramuscularly with 2 c.c. of the twenty-four-hour subculture used in the first control. Caseous abscesses were produced, limited to the areas of injection. Upon culture these yielded streptococci.

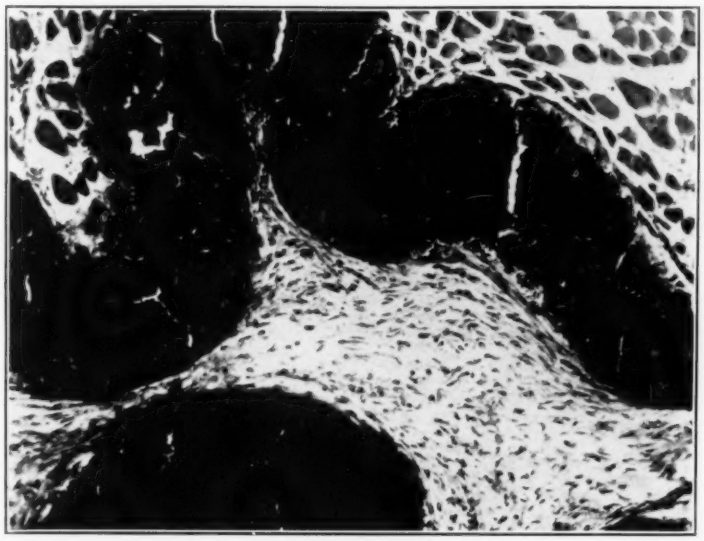


Fig. 3.—Skeletal muscle fibers in cross section are in the upper right and left hand corners. The remainder of the field is filled with large amorphous masses of calcareous material between and beneath which is invading fibrous tissue (hematoxylin and eosin stain).

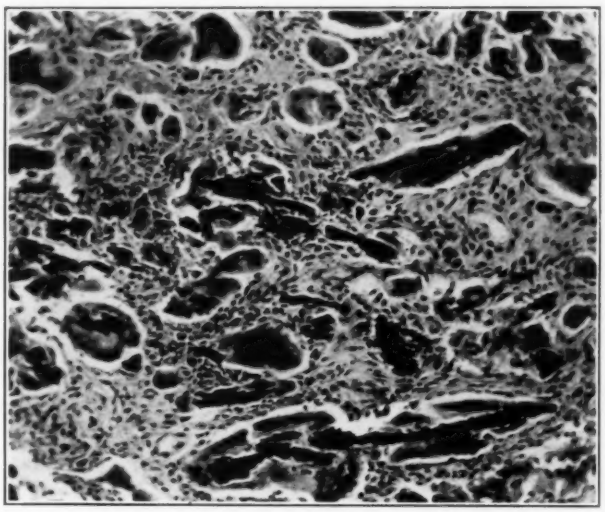


Fig. 4.—The lesion is in the process of resolution. Invading fibroblasts and foreign body giant cells are surrounding and breaking up the calcareous particles (hematoxylin and eosin stain).

A fourth group of rabbits was injected with streptococci intravenously and calcium gluconate intramuscularly. Twenty-four hours later the injected muscle and overlying skin were hot, swollen, and tender. But generally the animals did not sicken. Microscopic study (hematoxylin and eosin stain) showed loss of striation, swelling, round-cell reaction, and marked increase in the number of muscle-cell nuclei (Fig. 1). After three to fifteen days a grossly appreciable hardening could be made out. Cultures from the blood stream were sterile. Movement of the part was impaired. Following lethal anes-

thesia, necropsy revealed a roughened, hard, bonelike, yellow-white plaque which could be cut with difficulty and had a gritty feel (Fig. 2). Culture therefrom yielded only streptococci. All viscera were normal. Large, amorphous masses of calcareous material (staining blue with hematoxylin) appeared within the muscle. An increase of round cells, concentrated perivascularly, persisted, and mild fibroblastic invasion within and about the amorphous masses was apparent (Fig. 3). Diplococci and a few short-chain streptococci could be stained within these areas (Giemsa's method). Deposition of calcium attained a maximum in about ten days after initial inoculation.

Resolution was complete in three to six months. Invading fibroblasts and foreign body giant cells apparently surrounded, broke up, and removed the calcareous masses (Fig. 4). With disappearance of the foreign body giant cells, the final picture was a small amount of scar.

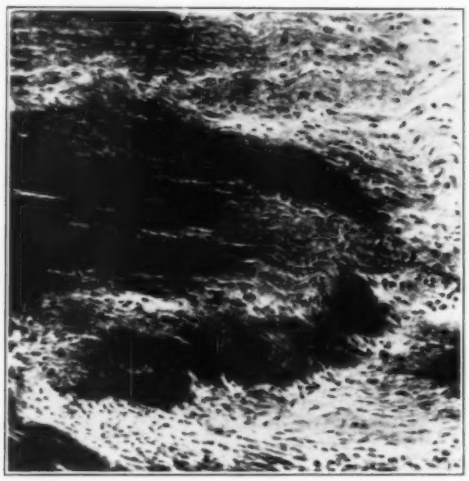


Fig. 5.—In one instance there was an attempt at bone formation. The calcium is being laid down in fine fibrils between which there is a lining up of osteoblast-like cells (hematoxylin and eosin stain).

In one instance after thirty-two days, a definite attempt at organized bone formation was observed, the calcium being laid down in fine fibrils associated with a lining up of osteoblast-like cells (Fig. 5).

SUMMARY

Streptococci recovered from an infected tooth were injected intravenously into rabbits. While one set of controls died of generalized infection, no specific localization could be obtained in a second. But such localization in muscles and connective tissues was made easy if simultaneous injection of calcium gluconate was practiced. Calcified lesions, ultimately absorbed, were produced except once, when what appeared to be bone was observed.

REFERENCES

1. Rosenow, E. C.: (a) Elective Localization of Streptococci, J. A. M. A. **65**: 1687, 1915. (b) Jordan, E. O., and Falk, I. S.: Newer Knowledge of Bacteriology and Immunology, Chicago, 1928, Univ. Chicago Press, pp. 576-589.
2. Haden, R. L.: Elective Localization in Eye of Bacteria From Infected Teeth, Arch. Int. Med. **32**: 828, 1923.

3. Nickel, A. C.: Elective Localization in Animals of Bacteria Isolated in Foci of Infection, J. A. M. A. **87**: 1117, 1926.
4. Rosenow, E. C.: Newer Knowledge of Bacteriology and Immunology by Jordan and Falk, Chicago, 1928, Univ. Chicago Press, p. 580.
5. Delitala, P.: E'agevole provocare l'organotropismo microbico? Ricerche sperimentali, Rassegna internaz. di clin. e terap. **17**: 1062, 1936.
6. Rhodes, G. B., and Apfelbach, C. W.: Chronic Localized Streptococcus Infections in Dogs, J. Infect. Dis. **43**: 215, 1928.
7. Rossides, T. N.: Use of Calcium Chloride in Formation of Experimental Staphylococcus Abscesses, Arch. Path. & Lab. Med. **3**: 1008, 1927.
8. Heubner, W., and Sanders, E.: Über die lokale Wirkung des 20proz. Calcium Sandoz (Calcium-Gluconate-Lactobionat), Klin. Wchnschr. **16**: 1650, 1937.
9. Goldman, Leon: Personal communication.
10. Herrold, R. D.: Treatment of Acute Complications of Gonococcus and Post-Gonococcus Infections With Calcium Gluconate, Ven. Dis. Inform. **11**: 55, 1930.
11. Oriani, F., Thoné, and Zau, Z. D., in Cantarow, A.: Calcium Metabolism and Calcium Therapy, Philadelphia, 1931, Lea & Febiger, p. 194.
12. von Hofe, F. H., and Jennings, R. E.: Calcium Deposition Following Intramuscular Administration of Calcium Gluconate. Report of Case in New-Born Infant, J. Pediat. **8**: 348, 1936.
13. Tumpeer, I. H., and Denenholz, E. J.: Calcium Deposit Following Therapeutic Injection in Tetany of New-Born, Arch. Pediat. **53**: 215, 1936.
14. Shands, A. R., Jr.: Studies in Bone Formation: Effect of Local Presence of Calcium Salts on Osteogenesis, J. Bone & Joint Surg. **19**: 1065, 1937.

THE BASAL METABOLIC RATE OF NORMAL INDIVIDUALS IN NEW ORLEANS*

A. G. Eaton, Ph.D., New Orleans, La.

THE lowered basal metabolic rate of normal individuals living in warm climates has already been the subject of several investigations. It is not the purpose of this paper to set up another set of standards in a field which is at the present overburdened with them, but rather to extend the work of others to less specific groups and to widen the age range. As a practical result of the work we have arrived at a set of corrections which may be applied to existing standards in predicting the basal heat production of individuals in New Orleans and, it is hoped, in other parts of the deep South.

The lowered basal metabolic rate for warm climates has been reported by de Almeida[1] in Brazil, Mukherjee and Gupta[2] in Calcutta, Mason and Benedict[3] in south India, Coons[4] in Oklahoma, Tilt[5] in Florida, Remington and Culp[6] in South Carolina, and Hindmarsh[7] in Australia. Conversely, Heinbecker[8, 9] has found the basal metabolic rate of the Eskimo, living in a cold climate, to be high.

EXPERIMENTAL

All of the subjects used in this investigation were white and of the so-called middle class. They consisted of students and staff of the School of

*From the Department of Physiology, Louisiana State University, School of Medicine, New Orleans.
Received for publication, February 16, 1939.

TABLE I

BASAL METABOLIC RATES OF WOMEN IN NEW ORLEANS

SUBJECT NO.	AGE	HT. CM.	WT. KG.	R. Q.	CAL./SQ. M./HR.	PERCENTAGE DEVIATION FROM STANDARD			
						SAGE STD.	H. & B. STD.	DREYER STD.	MAYO STD.
1	15	165.0	46.3	0.815	35.1	-18.4		- 6.9	-11.6
2	15	163.5	52.1	0.835	33.3	-22.7		-12.7	-16.1
Average				0.825	34.2	-20.5		- 9.8	-13.9
3	17	162.5	42.7	0.771	36.9	- 7.8		- 1.0	- 1.9
4	18	163.5	48.2	0.819	34.7	- 8.7		- 6.3	- 6.2
5	18	152.5	44.9	0.821	34.4	- 9.5		-10.9	- 7.0
6	18	163.5	48.4	0.852	37.5	- 1.3		+ 1.1	+ 1.3
Average				0.831	35.8	- 9.8		- 8.5	- 6.6
7	19	165.0	43.4	0.812	33.7	- 8.9		- 6.7	- 7.9
8	19	170.0	46.7	0.941	32.7	-13.9		- 7.8	-10.7
9	19	162.0	58.0	0.723	34.9	- 8.2		- 7.2	- 4.6
10	19	166.0	54.3	0.821	34.2	-10.0		- 7.2	- 6.6
Average				0.826	33.9	-10.3		- 7.2	- 7.5
11	20	160.5	65.1	0.812	30.4	-17.8	-17.7	-18.9	-16.2
12	20	168.5	53.0	0.846	32.7	-11.6	-11.2	- 9.4	- 9.9
13	20	162.0	50.5	0.794	37.1	- 0.0	+ 0.6	+ 1.3	+ 2.2
14	20	160.5	47.8	0.816	34.3	- 7.3	- 8.3	- 7.6	- 5.5
15	20	167.0	61.5	0.815	31.9	-13.8	-12.3	-13.3	-12.1
16	20	175.0	56.0	0.806	36.4	- 1.6	+ 3.2	+ 3.5	+ 0.3
17	21	170.5	60.9	0.765	34.1	- 7.8	- 4.8	- 5.1	- 5.8
18	21	166.0	48.3	0.862	34.1	- 8.0	- 6.9	- 5.1	- 5.8
19	22	162.5	57.5	0.797	30.4	-17.8	-17.2	-17.5	-15.8
20	22	160.0	51.4	0.789	33.1	-10.5	-10.0	- 9.2	- 8.3
21	22	160.6	51.0	0.800	34.4	- 7.0	- 6.8	- 6.1	- 4.7
22	22	149.8	41.6	0.839	30.0	-18.9	-22.6	-20.6	-16.9
23	22	152.5	49.8	0.817	33.0	-10.8	-16.1	-16.1	- 8.6
24	22	163.5	40.8	0.864	33.9	- 8.4	- 9.9	- 5.4	- 6.1
25	23	159.0	53.0	0.780	35.8	- 3.2	- 4.4	- 4.1	- 0.8
26	23	166.0	57.5	0.806	39.2	+ 5.9	+ 8.5	+ 9.0	+ 8.6
27	23	150.5	40.1	0.809	35.7	- 4.8	- 8.0	- 4.6	- 1.1
28	23	168.5	52.1	0.784	33.9	- 8.4	- 5.4	- 3.7	- 6.1
29	23	155.0	42.8	0.877	37.2	+ 0.8	- 1.7	+ 1.2	+ 3.1
30	24	168.5	57.5	0.843	32.5	-12.2	- 8.9	- 8.0	- 9.7
31	24	165.0	49.2	0.798	27.3	-26.2	-25.6	-23.6	-24.2
32	24	157.7	43.4	0.785	32.6	-11.9	-12.5	- 9.6	- 9.4
33	24	157.0	46.6	0.800	29.9	-19.2	-18.5	-16.9	-17.0
34	24	162.5	57.0	0.790	31.3	-15.4	-13.6	-13.4	-13.1
35	24	157.7	49.4	0.816	30.8	-16.8	-17.0	-15.9	-14.4
36	25	170.2	48.0	0.745	30.1	-18.7	-15.3	-12.1	-16.4
37	25	166.0	55.1	0.807	33.5	- 9.4	- 6.3	- 5.1	- 6.9
38	25	172.0	48.0	0.800	30.3	-18.1	-14.2	-10.8	-15.8
39	25	156.3	44.4	0.761	35.7	- 3.5	- 4.1	- 1.3	- 0.8
40	25	171.0	66.0	0.784	32.6	-11.9	- 7.3	- 7.1	- 9.4
Average				0.810	33.1	-10.7	-10.3	- 9.0	- 8.7
41	26	167.0	46.7	0.912	37.2	+ 0.5	+ 3.0	+ 6.9	+ 3.6
42	26	168.0	78.5	0.762	33.5	- 9.4	- 8.3	- 9.1	- 6.7
43	26	163.3	52.2	0.867	31.6	-14.6	-12.4	-10.9	-11.9
44	26	166.0	51.8	0.802	36.2	- 2.2	+ 1.6	+ 3.9	+ 0.8
45	26	170.4	52.9	0.814	31.4	-15.1	-11.1	- 8.8	-12.5
46	26	160.6	44.9	0.783	35.6	- 3.8	- 2.9	+ 0.4	- 0.8
47	27	163.4	56.5	0.782	30.5	-17.6	-10.4	- 9.5	-15.0
48	27	179.8	59.5	0.760	34.4	- 7.0	- 0.2	+ 2.4	- 4.2
49	27	160.5	49.8	0.799	36.5	- 1.4	+ 0.3	+ 2.4	+ 1.7

TABLE I—CONT'D

SUBJECT NO.	AGE	HT. CM.	KG. WT.	R. Q.	CAL./SQ. M./HR.	PERCENTAGE DEVIATION FROM STANDARD			
						SAGE STD.	H. & B. STD.	DREYER STD.	MAYO STD.
50	27	168.0	52.1	0.794	31.4	-15.1	-11.5	- 9.3	-12.5
51	28	173.5	63.7	0.772	33.2	-10.3	- 3.6	- 2.2	- 7.5
52	28	157.0	48.5	0.789	32.8	-11.4	-10.0	- 8.1	- 8.6
53	28	160.5	50.3	0.879	31.6	-14.6	-12.6	-10.9	-11.9
54	28	160.5	46.7	0.869	31.8	-14.1	-12.8	-10.0	-11.4
55	29	166.0	60.0	0.716	30.8	-16.8	-16.7	-15.9	-14.2
56	29	166.0	53.8	0.892	33.3	-10.0	- 5.9	- 3.8	- 7.2
57	29	165.0	51.3	0.867	29.8	-19.5	-16.5	-14.3	-17.0
58	29	165.0	49.0	0.818	33.3	-10.0	- 6.7	- 3.7	- 7.2
59	29	161.9	63.6	0.821	30.8	-16.6	-13.6	-13.6	-14.2
Average				0.816	32.9	-11.0	- 8.1	- 6.9	- 8.0
60	30	164.0	68.6	0.884	32.9	-11.1	- 6.4	- 6.5	- 8.1
61	30	149.8	37.8	0.847	36.7	- 0.8	- 5.4	- 0.2	+ 2.5
62	30	149.9	56.5	0.797	37.3	+ 0.8	+ 7.1	+ 5.7	+ 4.2
63	31	152.5	50.8	0.795	28.9	-20.8	-23.1	-20.7	-19.3
64	33	155.0	49.8	0.782	32.5	-10.9	- 9.1	- 8.3	- 9.0
65	33	149.8	60.5	0.750	34.2	- 6.3	- 0.8	+ 1.6	- 4.2
66	33	157.6	48.9	0.850	36.9	+ 1.1	+ 2.6	+ 5.2	+ 3.4
67	33	159.0	61.5	0.758	34.1	- 6.6	- 4.1	- 3.8	- 4.5
68	34	162.0	63.3	0.831	32.0	-12.3	- 9.1	- 8.5	-10.4
69	34	162.0	63.8	0.819	36.9	+ 1.1	+ 5.1	+ 5.6	+ 3.4
70	35	162.5	56.0	0.773	29.4	-19.4	-16.1	-14.7	-17.6
71	35	158.0	74.7	0.772	30.7	-15.9	-12.8	-13.3	-14.0
72	35	173.5	81.5	0.842	30.7	-15.9	- 9.7	- 8.8	-14.0
Average				0.808	33.3	- 9.2	- 7.4	- 6.6	- 7.8
73	36	163.3	54.7	0.917	33.4	- 5.8	- 4.0	- 2.2	- 6.2
74	36	160.5	65.5	0.754	30.2	-17.3	-14.1	-14.1	-15.2
75	36	167.0	60.9	0.720	30.3	-17.0	-12.5	-11.1	-14.9
76	37	152.0	42.5	0.942	33.1	- 9.3	- 8.1	- 5.0	- 7.0
77	37	165.3	96.2	0.842	32.5	-11.0	- 8.0	- 7.6	- 8.7
78	37	162.6	54.5	0.852	32.8	-10.1	- 7.0	- 3.9	- 7.9
79	38	157.6	82.6	0.803	36.5	- 0.0	+ 1.7	+ 0.9	+ 2.5
Average				0.833	32.7	-10.1	- 7.7	- 6.3	- 8.6
80	40	157.6	54.3	0.826	35.1	- 2.5	+ 1.6	+ 2.6	- 1.0
81	40	163.2	75.1	0.840	31.2	-13.3	- 9.3	- 9.2	-12.1
82	40	167.0	61.5	0.734	33.1	- 8.1	- 3.3	+ 1.9	- 6.8
83	42	162.0	63.3	0.801	35.2	- 2.1	+ 4.8	+ 5.4	- 0.8
84	42	168.5	61.9	0.970	31.9	-11.4	- 5.6	- 4.0	-10.1
85	43	166.0	60.5	0.768	35.0	- 2.9	+ 4.0	+ 5.5	- 1.1
86	44	165.0	63.7	0.834	31.2	-13.3	- 7.7	- 6.9	-11.9
87	46	162.0	47.6	0.878	30.0	-16.7	-11.0	- 9.0	-14.5
88	48	166.0	59.9	0.748	31.0	-13.9	- 6.2	- 5.1	-10.7
Average				0.822	32.6	- 9.1	- 4.8	- 3.8	- 7.7
89	50	164.7	63.3	0.816	33.3	- 4.9	+ 0.8	+ 1.3	- 3.2
90	51	162.0	83.1	0.839	29.5	-15.7	-12.8	-12.9	-13.7
91	51	157.6	56.1	0.811	33.9	- 3.1	+ 2.0	+ 2.2	- 0.9
92	51	167.5	89.2	0.957	34.6	- 1.1	+ 3.2	+ 3.9	+ 1.1
93	52	160.5	45.8	0.766	30.3	-13.4	-17.2	-15.3	-10.9
94	53	157.5	57.0	0.810	32.4	- 7.4	- 1.7	- 2.0	- 4.1
95	55	167.0	58.5	0.804	35.7	+ 2.0	+10.8	+11.7	+ 6.9
96	55	154.0	39.6	0.803	35.9	+ 2.6	+ 7.1	+10.0	+ 7.5
97	57	165.0	75.6	0.774	29.1	-16.9	- 7.9	- 8.2	-12.3
98	57	160.0	47.3	0.722	33.7	- 3.7	+ 7.1	+ 5.7	+ 1.5
Average				0.800	32.8	- 6.6	- 4.0	- 3.8	- 4.5

Medicine of Louisiana State University, and their friends and relatives, as well as patients of staff members. None exhibited endocrine disturbances. Examination of the data might seem to indicate the inclusion of a few borderline cases in both directions, but clinical support of this view is lacking. All of the individuals were in good health. They either had lived in this region all of their lives or had been here long enough to become acclimated.

The subjects came to the laboratory early in the morning, at least fourteen hours after the last meal. They rested at least forty-five minutes on a comfortable bed in a quiet room which was maintained at a comfortable temperature. Expired air was collected for a ten-minute period in a Tissot spirometer and analyses were made by means of a very accurate Haldane analyzer, frequently checked against outdoor air. All subjects showed normal body temperatures. Each subject was examined at least twice, on different days.

The values appearing in the tables are, for the most part, first determinations as used by Boothby, Berkson, and Dunn.[10] In a few cases the subjects exhibited restlessness, and in these individuals the first determination which showed constant pulse and respiratory rates was used. This did not occur often and the second determination was almost invariably satisfactory. In this manner the effect of training is largely ruled out, as well as the effect of nervousness.

The values reported here are not so low as those reported for New Orleans by Hakfesbring and Borgstrom[11] several years ago, which is to be expected, since their experiments were concerned entirely with well-trained subjects belonging to a selected group. Heat production was calculated from the total respiratory quotient, and no urinary nitrogen determinations were made due to the difficulty of accurate collection of twenty-four-hour samples when subjects are not maintained in a hospital. Values in the tables are expressed in terms of Calories per square meter per hour, and also in terms of percentage variation from the original Sage (Aub and DuBois) standard,[12] the Harris and Benedict standard multiple prediction tables,[13] the Dreyer standards (according to Stoner[14]), and the Mayo Foundation standards (Boothby, Berkson, and Dunn.[10]

Women.—Examination of the tables shows clearly that all four standards are too high for our subjects. Of the 98 women studied only 8 are as high as or higher than the Sage standards, while only 46 fall within the +10 per cent range. Only 5 are above +1 per cent, and the highest is +5.9 per cent. It is clear that the Sage standards would give valid prediction values in less than half of the women studied. The average deviation of all of the women studied is –9.5 per cent from the Sage standards. This is reasonably uniform for all age groups except in the 50-60 year classification, where it is only –6.6 per cent. This latter is a small group and not too much emphasis can be placed upon it. Applying a single correction by deducting 9.5 per cent from the Sage standards, one then finds that 48 of the 98 individuals show values as high as or higher than the revised standard and 50 subjects fall below. There

are 8 subjects who exceed the +10 per cent, 4 of them by a fraction of a per cent and only one slightly more than 15 per cent (15.4). Three subjects fall below the −10 per cent and of these, one below the −15 per cent (16.7). Thus, with this one simple correction, uniform for all ages, one may accurately predict the basal metabolic rate of more than 90 per cent of the individuals studied, and the remainder become borderline cases. The standard deviation has been calculated for each series as a whole from the percentage deviation from each revised standard as a basis. This method was used since some of the groups were too small to allow for the estimation of a valid standard deviation. Calculated in this manner the standard deviation is 6.778 per cent or 2.25 Calories. The probable error derived from the standard deviation is ±0.45.

Our data fit the unrevised Harris and Benedict prediction tables slightly better. Here, because the original tables give no prediction values below age 20, only 88 women have been compared. About 22 per cent (19 women) show values as high as or higher than the tables, and 62 per cent (55 subjects) fall within the ±10 per cent range. It is not at all possible to apply a single correction to this standard since different age groups show such variable deviation from the standard.

The data for women in this series fit the Dreyer standard better than any of the other three. Of the subjects tested 23 have basal metabolic rates as high as or higher than the standard, and 72 fall within the ±10 per cent range. Again, as is the case with the Harris Benedict prediction tables, a single correction factor for use over the entire age range cannot be applied.

The Mayo Foundation standards, while in general slightly lower than the Sage standards for women, are also much too high in this climate. Only 14 individuals show values as high as or higher than the standard and 60 fall within the ±10 per cent range. A single correction, the subtraction of 7.4 per cent from the standard, which is the average deviation of all cases, serves to bring 89 cases within the ±10 per cent range and leaves only 3 outside the ±15 per cent limit, and these but slightly. Considering all subjects, 50 fall below the corrected standard and 48 are above. This is about as good a revised list of standards as that obtained by deducting 9.5 per cent from the Sage tables. The standard deviation calculated as described above is 6.931 per cent or 2.30 Calories, with a probable error of ±0.472.

Men.—As is the case for the basal metabolic rates for women, our data show that the standards are all too high for this locality. Only 3 of the 62 men studied have basal metabolic rates as high as the Aub and DuBois standards, and only 30 are within the ±10 per cent range. If a correction factor of 10 per cent is added to all values, 32 individuals are below the revised standard and 30 are above; only 2 are more than 10 per cent below, and 3 show rates of more than 10 per cent. No individual exceeds ±15 per cent. The standard deviation is 5.773 per cent or 2.07 Calories, and the probable error is ±0.495.

Only 5 values exceed the Harris and Benedict prediction table, while 33 fall within the ± 10 per cent range. The fit with the Dreyer standards is somewhat better where 5 values are above those of the tables and 42 fall within the ±10 per cent range.

TABLE II

BASAL METABOLIC RATES OF MEN IN NEW ORLEANS

SUBJECT NO.	AGE	HT. CM.	WT. KG.	R. Q.	CAL./SQ. M./HR.	PERCENTAGE DEVIATION FROM STANDARD			
						SAGE STD.	H. & B. STD.	DREYER STD.	MAYO STD.
1	15	171.0	49.6	0.822	40.2	-12.6	- 0.2	- 2.1	-12.8
2	16	160.5	58.5	0.821	42.4	- 3.6	+ 4.7	- 0.5	- 7.0
3	16	178.5	56.5	0.812	38.8	- 9.7	- 4.0	- 1.1	-14.7
Average				0.817	40.6	- 6.7	+ 0.4	- 0.8	-10.4
4	18	174.0	60.4	0.802	38.0	- 7.3	- 5.2	- 4.7	-11.4
5	19	187.0	77.9	0.858	38.8	- 5.4	- 3.5	- 1.1	- 8.1
6	19	173.5	68.7	0.796	41.4	+ 0.9	+ 2.5	- 0.0	- 1.9
7	19	183.2	70.4	0.832	39.0	- 4.9	- 1.9	+ 1.2	- 7.6
8	19	183.0	66.0	0.767	35.1	-14.4	-11.6	- 8.9	-17.1
9	19	172.0	65.1	0.817	36.8	-10.2	- 7.8	- 8.0	-12.8
10	19	167.0	59.5	0.789	38.0	- 7.3	- 4.8	- 6.7	-10.0
11	19	172.3	65.1	0.804	36.2	-11.7	-12.3	-12.5	-14.2
12	19	176.0	78.9	0.782	37.7	- 8.0	- 8.2	- 6.2	-10.7
Average				0.805	37.9	- 8.7	- 6.9	- 6.0	-11.7
13	20	175.0	63.3	0.826	38.9	- 1.5	- 1.6	- 1.1	- 6.5
14	20	176.0	73.3	0.806	37.2	- 9.3	- 9.2	- 7.7	-10.6
15	21	166.0	66.5	0.874	33.9	-14.2	-15.3	-16.5	-17.7
16	21	169.5	61.9	0.786	37.1	- 6.1	- 7.3	- 7.8	- 9.9
17	21	178.5	96.2	0.864	35.5	-10.1	-14.7	-10.3	-13.8
18	21	168.9	63.6	0.817	32.6	-17.5	-18.1	-18.7	-20.9
19	22	173.5	57.4	0.763	36.5	- 7.6	- 4.9	- 4.2	-10.8
20	22	179.1	68.2	0.865	34.4	-12.9	-12.7	-10.3	-15.9
21	22	176.0	63.3	0.833	33.1	-16.2	-15.2	-13.8	-19.1
22	22	176.0	67.3	0.825	40.8	+ 3.3	+ 4.5	+ 6.5	- 0.2
23	22	176.0	72.5	0.783	39.0	- 1.2	- 0.3	- 0.6	- 4.6
24	23	176.0	82.3	0.790	32.0	-16.5	-21.0	-18.3	-21.1
25	23	185.6	73.0	0.835	34.8	-11.9	-11.6	- 7.0	-14.9
26	23	175.0	89.7	0.789	37.1	- 6.1	-10.0	- 6.8	- 8.8
27	23	178.5	69.1	0.785	33.4	-15.4	-15.5	-13.0	-18.0
28	24	168.0	50.8	0.790	32.1	-18.7	-16.1	-16.8	-20.7
29	24	172.1	67.5	0.802	34.4	-12.9	-13.2	-12.3	-15.1
30	24	176.0	58.0	0.863	34.5	-12.6	-10.9	- 9.3	-14.8
31	24	181.0	81.0	0.795	36.6	- 7.3	- 8.3	- 3.9	- 9.6
32	24	180.0	92.7	0.888	36.1	- 9.0	-11.6	- 6.5	-10.9
33	24	175.3	72.7	0.810	36.6	- 7.3	- 7.9	- 5.6	- 9.6
34	25	175.5	73.8	0.790	34.2	-13.4	-14.2	-11.8	-15.1
35	25	174.6	60.9	0.785	35.4	-10.4	- 8.5	- 7.2	-12.2
36	25	181.0	71.2	0.846	37.8	- 4.3	- 6.6	- 2.6	- 6.2
37	25	171.0	64.2	0.904	37.2	- 5.8	- 5.0	- 4.3	- 7.7
38	25	180.0	71.7	0.830	33.4	-15.4	-15.5	-12.3	-17.1
39	25	178.5	72.5	0.782	29.7	-24.8	-24.5	-21.3	-26.3
40	25	172.0	82.5	0.858	34.2	-13.7	-15.8	-13.5	-15.1
41	25	181.0	68.2	0.915	35.4	-10.4	- 9.3	- 5.8	-12.2
Average				0.828	35.3	-10.8	-11.2	- 9.3	-13.2
42	26	185.7	97.6	0.827	36.2	- 8.3	-15.9	- 8.2	- 9.8
43	26	182.0	65.5	0.845	32.5	-17.7	-16.1	-12.6	-19.0
44	27	185.0	77.9	0.822	37.0	- 5.0	- 8.0	- 2.3	- 7.5
45	27	170.0	69.9	0.807	36.4	- 7.8	- 7.3	- 6.4	- 9.0
46	27	181.0	62.5	0.839	34.9	-11.7	- 8.6	- 5.0	-10.2
47	27	167.0	56.5	0.804	32.2	-18.2	-20.5	-21.1	-19.5
48	27	171.0	70.7	0.773	30.3	-23.3	-22.6	-21.6	-24.2
49	28	173.5	83.6	0.948	37.5	- 5.6	- 6.2	- 2.9	- 6.0
50	28	180.0	76.5	0.794	35.6	- 9.8	-11.2	- 7.0	-10.8
51	29	180.0	78.9	0.853	34.2	-13.4	-14.4	- 8.9	-13.9
Average				0.831	34.7	-12.2	-13.1	- 9.6	-13.0

TABLE II—CONT'D

SUBJECT NO.	AGE	HT. CM.	WT. KG.	R. Q.	CAL./SQ. M./HR.	PERCENTAGE DEVIATION FROM STANDARD			
						SAGE STD.	H. & B. STD.	DREYER STD.	MAYO STD.
52	31	175.0	83.3	0.778	34.1	-13.7	- 9.4	-11.0	-13.7
53	31	181.0	68.2	0.827	34.1	-13.7	-10.5	- 6.5	-13.7
54	32	172.0	82.1	0.782	40.0	+ 1.3	+ 1.3	+ 4.7	+ 1.8
55	32	164.5	65.1	0.804	36.0	- 8.9	- 6.2	- 7.1	- 8.4
56	33	174.0	70.5	0.810	32.7	-17.2	-18.0	-11.5	-16.6
Average				0.800	35.4	-10.7	- 8.8	- 7.2	-10.5
57	40	163.5	79.4	0.835	35.2	- 8.7	-10.3	- 9.8	- 7.3
58	43	171.0	85.5	0.780	30.8	-20.0	-17.9	-15.0	-18.7
59	43	179.5	81.5	0.818	37.7	- 2.1	+ 0.8	+ 5.9	- 0.5
60	44	178.5	69.0	0.829	34.4	-10.6	- 5.3	- 2.4	- 8.8
61	45	183.2	90.9	0.816	37.6	- 2.3	- 0.8	+ 6.0	- 0.0
62	46	171.0	72.5	0.819	33.5	-13.0	- 8.1	- 7.2	-10.7
Average				0.816	34.9	- 9.5	- 7.1	- 5.7	- 7.8

When the data for men are compared with the Mayo Foundation standards, only one of the 62 values is as high as or higher than the standard, and only 23 fall into the ±10 per cent range. Deducting 11 per cent from the standard, the agreement becomes excellent, 31 values being below the revised standard and 31 above. Now only 3 cases are below −10 per cent, with one of these more than −15 per cent (15.3); 4 exceed the +10 per cent limit, and none the +15. The standard deviation is 5.761 per cent or 2.06 Calories, and the probable error is ±0.494.

It would appear, then, on the basis of the data presented, that one may easily correct two widely used standards for use in New Orleans. Perhaps the simplest correction is to deduct 10 per cent from the original Aub and DuBois (Sage) standard for both men and women. In routine practice a few normal individuals will fall outside the ±10 per cent range. These should, no doubt, be considered borderline cases and must be compared with other standards. Table III gives correction values for these standards.

TABLE III

COMPARISON OF AVERAGE BASAL METABOLIC RATES IN NEW ORLEANS WITH THE MOST COMMONLY USED STANDARDS

AGES	MEN				WOMEN			
	SAGE STD.	H. & B. STD.	DREYER STD.	MAYO STD.	SAGE STD.	H. & B. STD.	DREYER STD.	MAYO STD.
18					- 9.8		- 8.5	-6.6
19	- 8.7	- 6.9	-6.0	-11.7	-10.3		- 7.2	-7.5
20-25	-10.8	-11.2	-9.3	-13.2	-10.7	-10.3	- 9.0	-8.7
25-30	-12.2	-13.1	-9.6	-13.0	-11.0	- 8.1	- 6.9	-8.0
30-40	-10.7	- 8.8	-7.2	-10.5	-10.1	- 7.7	- 6.3	-8.6
40-50	- 9.5	- 7.1	-5.7	- 6.7	- 9.1	- 4.8	- 3.8	-7.7
50-60					- 6.6	- 4.0	- 3.8	-4.5
Average	-10.2	- 9.4	-7.6	-11.0	- 9.5	- 5.0	- 6.4	-7.4

Equally valid predictions may be made by deducting 11 per cent from the Mayo Foundation standards for men and 7 per cent for women. It would appear from data presented that, using these standards, the basal metabolic rate

of men is reduced more in this locality than that of women. Our data are too meager to prove this conclusively. This difference, should it be substantiated with much more data, might be due to a lessened desire on the part of men for strenuous exercise in warm climates, whereas only a small proportion of women are very athletic in any climate.

The cause of the lowered basal metabolic rate has been discussed by many authors. It is a fact that metabolism is somewhat proportional to the average annual temperature, although the relationship cannot, as yet, be expressed as a linear function. This is to be expected so long as methods differ and various criteria are used. For instance, our values are 6 to 8 per cent higher than those of Hafkesbring and Borgstrom.[11] These authors used well-trained subjects, and the values expressed in our work are mostly first determinations similar to values obtained for clinical diagnosis.

The work of Borgstrom and Bost,[15] Borgstrom, Bost, and Hafkesbring,[16] and Borgstrom, Hafkesbring, and Bost[17] shows that the dietary in New Orleans is normally somewhat lower in respect to both Calories and proteins than that in northern cities. Although no evidence is available, one cannot help feeling that the present diet in New Orleans is higher in protein than formerly, with the increased efficiency of refrigeration. Sea food in particular, eggs, and meat have improved in quality so markedly that they furnish a very substantial part of the diet. The foregoing authors did not entirely succeed in showing the lowered diet to be the cause of the lower metabolic rate. It would seem more reasonable to regard the lowered dietary as only part of a generalized reaction to a warm climate and the lowered metabolic rate as a result of this generalized reaction. Lessened desire for strenuous physical exertion and greater ability and desire for relaxation fit into the same picture and must be considered as probable factors in lowering the metabolic rate. Whatever may be the cause, the lowered basal heat production lessens the strain on the heat-eliminating mechanism and makes life more comfortable in a subtropical climate, especially during hot humid nights, when heat is so difficult to dissipate. The mechanism by which this adaptation is brought about is as yet obscure.

The corrections proposed in this study are probably applicable to much of the deep South, since our data are in agreement with those of Remington and Culp[6] for medical students and nurses at Charleston, S. C., and with those of Tilt and Walters[18] for young college women in Florida. Whether our values could be applied to Oklahoma is somewhat problematic, although Coons[4] has arrived at approximately the same figures for college women. The diet commonly consumed in New Orleans is not as deficient as that reported by Coons and Schiefelbusch.[19]

SUMMARY

The basal metabolic rates of 98 women and 62 men in New Orleans have been compared with four commonly used standards and found to be lower than any of them. It is proposed to employ a correction factor of –10 per cent to all

values of the Aub and DuBois standards, or –11 per cent for men and –7 per cent for women to the recent Mayo Foundation standards. These corrections are shown to be valid statistically.

REFERENCES

1. de Almeida, A. O.: Le metabolisme de l'homme tropical, J. de physiol. et de path. gén. **22**: 12, 1922.
2. Mukherjee, H. N., and Gupta, P. C.: Basal Metabolism of Indians (Bengalis), Indian J. M. Research **18**: 807, 1931.
3. Mason, E. D., and Benedict, F. G.: The Basal Metabolism of South Indian Women, Indian J. M. Research **19**: 75, 1931.
4. Coons, C. M.: The Basal Metabolism of Oklahoma Women, Am. J. Physiol. **98**: 692, 1931.
5. Tilt, J.: The Basal Metabolism of Young College Women in Florida, J. Biol. Chem. **96**: 635, 1930.
6. Remington, R. E., and Culp, F. B.: Basal Metabolic Rate of Medical Students and Nurses in Training at Charleston, S. C., Arch. Int. Med. **47**: 366, 1931.
7. Hindmarsh, E. M.: Basal Metabolic Rate of Students in Sydney, N. S. W., With Discussion on Methods of Determining Basal Metabolism, Australian J. Exper. Biol. & M. Sc. **4**: 225, 1927.
8. Heinbecker, P.: Studies on Metabolism of Eskimos, J. Biol. Chem. **80**: 461, 1928.
9. Heinbecker, P.: Further Studies on Metabolism of Eskimos, J. Biol. Chem. **93**: 327, 1931.
10. Boothby, W. M., Berkson, J., and Dunn, H. L.: Studies of the Energy of Metabolism of Normal Individuals: A Standard for Basal Metabolism, With a Nomogram for Clinical Application, Am. J. Physiol. **116**: 468, 1936.
11. Hafkesbring, R., and Borgstrom, P.: Studies of Basal Metabolism in New Orleans, Am. J. Physiol. **79**: 221, 1926.
12. Aub, J. C., and DuBois, E. F.: The Metabolism of Old Men, Arch. Int. Med. **19**: 823, 1917.
13. Harris, J. A., and Benedict, F. G.: A Biometric Study of Basal Metabolism in Man, Carnegie Institution of Washington, Pub. No. 279, 1919.
14. Stoner, W. H.: Notes on Basal Metabolism. V. Tables of Values of Dreyer's Formulas, Boston Med. & Surg. J. **180**: 239, 1923.
15. Borgstrom, P., and Bost, R. W.: Protein Intake as Indicated by Urinary Nitrogen, Am. J. Physiol. **79**: 229, 1926.
16. Borgstrom, P., Bost, R. W., and Hafkesbring, R.: Dietary Studies in New Orleans in 1925, Am. J. Physiol. **79**: 237, 1926.
17. Borgstrom, P., Hafkesbring, R., and Bost, R. W.: Effect of Increased Diet on Basal Metabolism in New Orleans, Am. J. Physiol. **79**: 245, 1926.
18. Tilt, J., and Walters, C. F.: A Study of the Basal Metabolism and Diet of Normal Young College Women in Florida, J. Nutrition **9**: 109, 1935.
19. Coons, C. M., and Schiefelbusch, A. T.: The Diets of College Women in Relation to Their Basal Metabolism, J. Nutrition **5**: 459, 1932.

HEMOLYSIS PRODUCED BY STAPHYLOCOCCUS COLONIES AND TOXIN ON AGAR MEDIA CONTAINING VARIOUS ANIMAL BLOODS*

R. H. Rigdon, M.D., Nashville, Tenn.

MANY studies have been made since 1894 on the hemotoxin produced by staphylococci.[1-3] Washed red blood cell suspensions from different animals have usually been used for these studies. It is well known now that the corpuscles of different species show a variation in their susceptibility to staphylococcus toxin. Gross[4] gives the following order of susceptibility of the red blood cell from different species: rabbit, ox, sheep, goat, man, guinea pig, and horse. Rabbit red blood cells are the most susceptible and they have been more frequently used in the titration of staphylococcus toxin. They are used at this time in the standardization of staphylococcus antitoxin.

The hemolysis which occurs around certain colonies of staphylococci growing on the surface of blood agar plates has been studied by Baerthlein,[5] Naidu,[6] Rhodes,[7] Packalen[8] and others.

Little attention has been given to the selection of blood for the media in which staphylococci are grown. The effects produced by dropping staphylococcus toxin on the surface of blood agar plates also have not been adequately studied.[9] In the present experiment different strains of hemolytic staphylococci were placed on infusion agar containing blood from different species. Staphylococcus toxin was also dropped upon the surface of similar media to compare the lysis produced by it with that produced by staphylococci.

Hemolysis About Staphylococcus Colonies.—The type of blood used in agar is usually determined by the animals available in the laboratory. Little, if any, consideration is given to the selection of blood suitable for the differentiation of hemolytic and nonhemolytic strains of staphylococci. The following experiment was made to determine what influence various bloods might have on the formation of the hemolytic zone.

Blood was obtained from man, sheep, and rabbit and defibrinated with glass beads. A 5.0 per cent concentration of blood agar was prepared and 25 c.c. of this medium was put into Petri dishes. The surface of these plates was inoculated with young broth cultures of staphylococci isolated from the following sources:

Strain 13—Lung at autopsy. Bronchopneumonia.

Strain 14—Furuncle on neck.

Strain 16—Stock strain from this laboratory.

*From the Department of Pathology, Vanderbilt University Medical School, Nashville. Aided by a grant from the Division of Medical Sciences, Rockefeller Foundation.
Received for publication, February 16, 1939.

Strain A-O—Variant from culture isolated from pharynx of patient with agranulocytic angina.

Strain 34—Furuncle on arm.

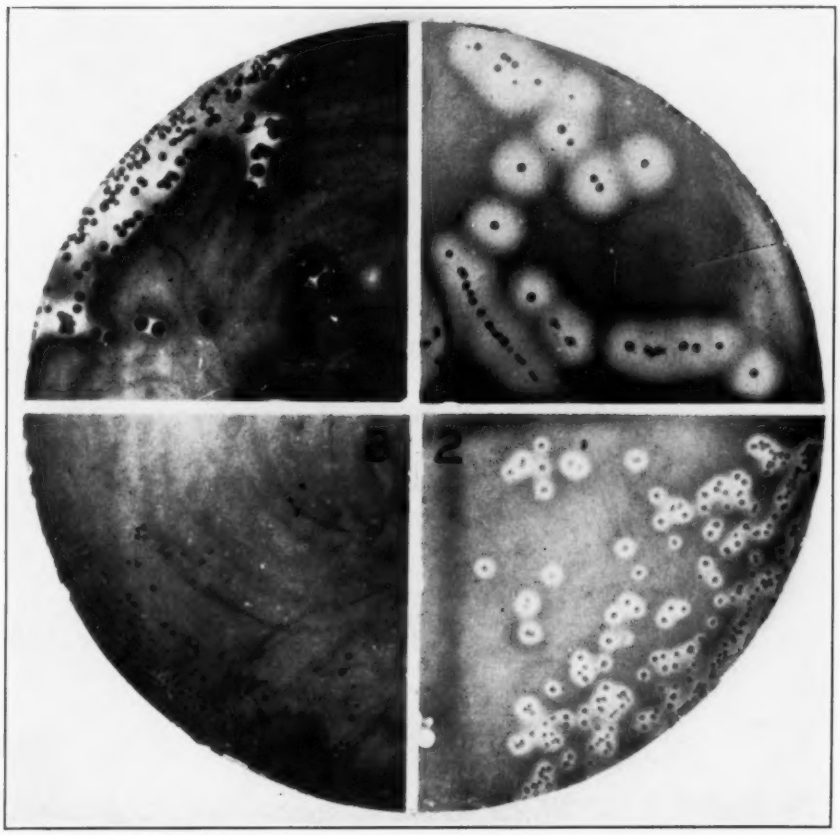


Fig. 1.—Colonies of staphylococci growing on blood agar plates. Segment *1*, rabbit blood; *2*, sheep blood; *3* and *4*, human blood. Segments 1, 2, and 3, photographed after twenty-four hours' incubation. Segment 4 is same as 3, photographed after ninety-six hours. Note the wide variation in the size of the hemolytic zone about the colonies on the different bloods.

Hemolysis was present after twenty-four hours around each colony of every culture growing on sheep and rabbit blood agar. No hemolysis was present on the plates containing human blood at this time. After forty-eight hours a narrow hemolytic zone was present around some of the colonies growing on the human blood agar. The width of the hemolytic zone was always much greater on the rabbit blood agar than on either of the other two types of media. The variations in the hemolytic zones are shown in Fig. 1. Segment 1 contains rabbit blood; segment 2, sheep blood; and segments 3 and 4, human blood. Each plate was inoculated at the same time with strain 34. Segments 1, 2, and 3 were photographed after twenty-four hours, and segment 4 after ninety-six hours.

The hemolysis produced by staphylococci grown under anaerobic conditions was studied. Broth cultures of staphylococci were added to 5.0 per cent rabbit blood agar and the media were poured into Petri dishes. The

surfaces of these plates were covered with vaseline. They were incubated at 37° C. for twenty-four hours. No hemolysis was present about any of the colonies at this time. The hemoglobin was slightly chocolate in color after forty-eight hours' incubation, but no lysis about any of the colonies had occurred. Blood agar plates containing the same cultures of staphylococci without the vaseline showed zones of hemolysis only about the surface colonies.

TABLE I

DEGREE OF HEMOLYSIS PRODUCED BY A SINGLE PREPARATION OF STAPHYLOCOCCUS TOXIN WHEN A 1.0 PER CENT CONCENTRATION OF RABBIT, SHEEP, AND HUMAN BLOOD CELLS IS USED

AMOUNT TOXIN IN C.C.	RED BLOOD CELLS		
	RABBIT	SHEEP	HUMAN
	DEGREE OF HEMOLYSIS*		
0.08	4	3	0
0.06	4	3	0
0.05	4	3	0
0.04	4	2	0
0.035	4	2	0
0.03	4	2	0
0.025	4	2	0
0.017	4	2	0
0.015	4	2	0
0.013	4	2	0
0.01	4	1	0
0.008	4	1	0
0.006	4	1	0
0.005	4	1	0
0.004	4	1	0
0.0035	4	1	0
0.03	4	1	0
0.0025	4	1	0
0.002	4	1	0
0.0017	3	0	0
0.0015	3	0	0
0.0013	3	0	0
0.001	3	0	0
0.0008	3	0	0
0.0006	3	0	0
0.0005	3	0	0
0.0004	3	0	0
0.00035	2	0	0
0.0003	2	0	0
0.00025	2	0	0
0.0002	2	0	0
0.00017	2	0	0
0.00015	1	0	0
0.00013	1	0	0
0.0001	0	0	0

*4 indicates complete lysis. 3 indicates 75 per cent lysis. 2 indicates 50 per cent lysis. 1 indicates 25 per cent lysis. 0 indicates no lysis.

Hemolysis Produced by Staphylococcus Toxin.—Blood from man, sheep, and rabbit was defibrinated and the cells were washed thoroughly four times with saline and used in a 1 per cent concentration for titration of the hemotoxin. The tubes were left at room temperature two hours and then placed in the ice-box overnight. Table I shows the degree of lysis produced by the same concentration of staphylococcus toxin with the different red blood cells. From these data it is evident that the rabbit red blood cell is most sensitive to this toxin. The sheep cell is much more resistant and the human cell is not lyzed at all by 0.8 c.c. of the toxin within eighteen hours.

A similar experiment was carried out using red blood cells from a larger number of species. The following is the order of increasing susceptibility of the red blood cells to the toxin: guinea pig, chicken, horse, man, sheep, turtle, monkey, mouse, dog, rat, pig, cat, cow, goat, and rabbit.

The following experiment was made to see if human red blood cells placed in infusion agar would resist the hemolytic effect of staphylococcus toxin. The remainder of the agar plates used for inoculating the different strains of staphylococci in the preceding experiment was used. The toxin was dropped through a 27 gauge needle onto the surface of these blood agar plates in the following dilutions: undiluted, 1:10, 1:50, 1:100, and 1:1,000. Table II shows the presence or absence of hemolysis on the different blood agar plates. The data given in this table show that no hemolysis is produced by this toxin when it is dropped on the surface of the plates containing human blood. Furthermore, less hemolysis occurs on the sheep blood agar than on the plates containing rabbit blood.

All the experiments indicate that the human red blood cell is more resistant to the hemolytic action of staphylococcus toxin than either the rabbit or the sheep cells. The sheep cell is also more resistant than the rabbit.

TABLE II

HEMOLYSIS PRODUCED BY STAPHYLOCOCCUS TOXIN ON BLOOD AGAR PLATES CONTAINING RED BLOOD CELLS OF DIFFERENT ANIMALS

	CONCENTRATION OF TOXIN AND PRESENCE OR ABSENCE OF LYSIS				
BLOOD	UNDILUTED TOXIN	1:10	1:50	1:100	1:1,000
Rabbit	+	+	+	+	+
Sheep	+	+	0	0	0
Human	0	0	0	0	0

The presence (+) or absence (0) of hemolysis after seventy-two hours.

Influence of Staphylococcus Antitoxin on the Hemolysis Occurring on Blood Agar Plates.—Natural staphylococcus antitoxin is known to occur in varying amounts in the serum of both man and laboratory animals. The variations in the degree of lysis of the red blood cells from different animals using the standard methods of titration apparently cannot be explained by the presence of natural antitoxin. The cells in such experiments are washed adequately to remove the greater portion of natural antitoxin. Furthermore, the cells are diluted sufficiently to render nil the action of any residual antitoxin. However, it is conceivable that defibrinated blood added to infusion agar might contain enough natural staphylococcus antitoxin to inhibit completely or delay the development of hemolysis about the colonies. The serum from human, sheep, and rabbit blood used in this experiment was, therefore, titrated for staphylococcus antihemolysin and each was found to contain essentially the same amount.

Standard staphylococcus antitoxin* was added to a group of rabbit blood agar plates. These were used to study the effect of the antitoxin on the

*Supplied by the National Institute of Health.

TABLE III

THE DEGREE OF HEMOLYSIS PRODUCED BY CULTURES OF STAPHYLOCOCCI ON AGAR PLATES CONTAINING DIFFERENT AMOUNTS OF STAPHYLOCOCCUS ANTITOXIN

STRAIN OF STAPHYLOCOCCUS	HEMOLYSIS AND AMOUNT OF ANTITOXIN IN THE PLATES						
PLATES	1	2	3	4	5	6	7
UNITS OF A.T.	0.4	1.2	2.0	4.0	8.0	20.0	CONTROL
10	+	+	+	+	+	0	+
13	+	+	+	0	0	0	+
14	+	+	+	+	+	+	+
15	+	+	+	+	+	0	+
16	0	0	0	0	0	0	+
34	+	+	+	0	0	0	+
A - O	+	+	+	+	+	0	+

(+) Presence, (0) absence of hemolysis after twenty-four hours' incubation at 37°C.

PLATE	ANTITOXIN UNITS PER PLATE
1	0.4
2	1.2
3	2.0
4	4.0
5	8.0
6	20.0
7	0.0

TABLE IV

HEMOLYSIS PRODUCED BY STAPHYLOCOCCUS TOXIN ON RABBIT BLOOD AGAR PLATES CONTAINING DIFFERENT AMOUNTS OF ANTITOXIN

(A series of dilutions of the toxin was dropped on the surface of these plates)

ANTITOXIN IN BLOOD AGAR PLATE	TIME IN HOURS*	DILUTION OF TOXIN							
		1:10	1:30	1:50	1:60	1:70	1:80	1:90	1:100
1	2	+	+	+	+	+	+	+	+
0.4 unit	4	+	+	+	+	+	+	+	+
	18	+	+	+	+	+	+	+	+
2	2	+	+	+	+	+	+	0	0
1.2 units	4	+	+	+	+	+	+	+	+
	18	+	+	+	+	+	+	+	+
3	2	+	+	+	+	+	+	0	0
2.0 units	4	+	+	+	+	+	+	+	+
	18	+	+	+	+	+	+	+	0
4	2	+	+	0	0	0	0	0	0
4.0 units	4	+	+	0	0	0	0	0	0
	18	+	+	0	0	0	0	0	0
5	2	+	0	0	0	0	0	0	0
8.0 units	4	+	0	0	0	0	0	0	0
	18	+	0	0	0	0	0	0	0
6	2	0	0	0	0	0	0	0	0
20.0 units	4	+	0	0	0	0	0	0	0
	18	+	0	0	0	0	0	0	0
7	2	+	+	+	+	+	+	+	+
Control	4	+	+	+	+	+	+	+	+
	18	+	+	+	+	+	+	+	+

*Time refers to two hours after incubation and four and eighteen hours after being placed in icebox.

+, presence of hemolysis; 0, absence.

hemolysis which occurs around the colonies and that produced by a drop of toxin on the surface of the same media. The following amounts of antitoxin were added to the infusion agar at 40 to 45° C. immediately before the defibrinated rabbit blood. A 5.0 per cent concentration of blood was used in the media.

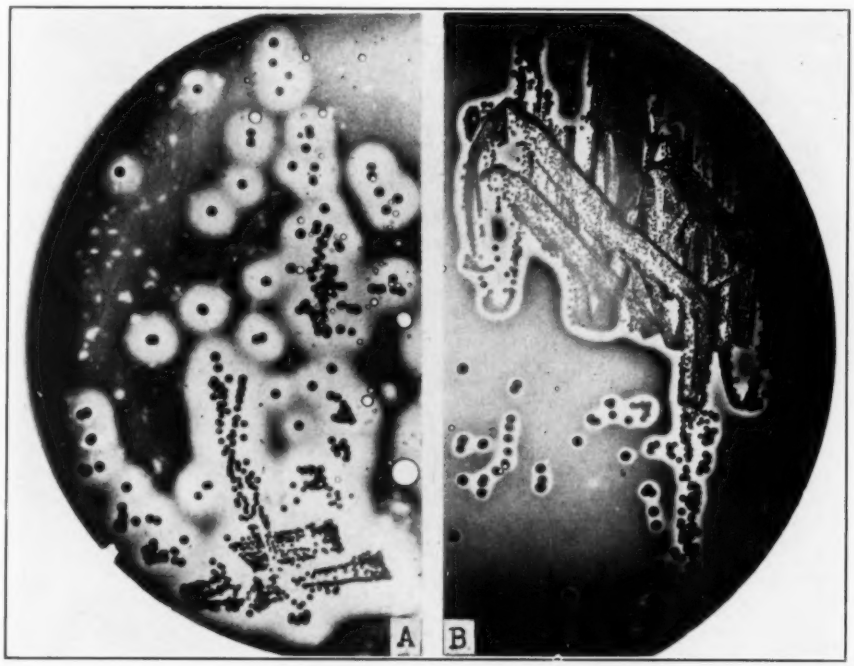


Fig. 2.—*A*, This agar plate was made with 5 per cent rabbit blood. *B* was prepared in the same manner, except 2 units of staphylococcus antitoxin were added immediately before the medium was poured into the Petri dish. The two plates were inoculated with the same strain of staphylococci and were photographed after twenty-four hours' incubation. The hemolytic zone is smaller in the plate containing the antitoxin. The size of the individual colonies is approximately the same.

The cultures of hemolytic staphylococci used for inoculating the above plates were all pigmented. They were the same as those used previously in this study. Strains 10 and 15 were also included in the present collection. The former was obtained at autopsy from a case of staphylococcus septicemia and the latter was isolated from a furuncle on the arm. The cultures were grown in infusion broth for eighteen hours immediately preceding the plate inoculation.

A record of the presence or absence of hemolysis about the individual colonies after twenty-four hours' incubation is shown in Table III. There is a variation in the width of the zone about similar colonies on the different blood agar plates as shown in Fig. 2.

The colonies from strain 16 failed to show any hemolysis after twenty-four hours' incubation at 37° C. on the plate containing 0.4 unit of antitoxin. A narrow zone of lysis was present, however, after the plate remained in the icebox for seventy-two hours. The zone of hemolysis about the colonies from

the other strains of staphylococci also increased during the time they were in the icebox. No quantitative record of the hemolysis was made in this study.

Different dilutions of staphylococcus toxin were dropped on the surface of some of the rabbit blood agar plates containing the different amounts of staphylococcus antitoxin to see if hemolysis would be inhibited here by the

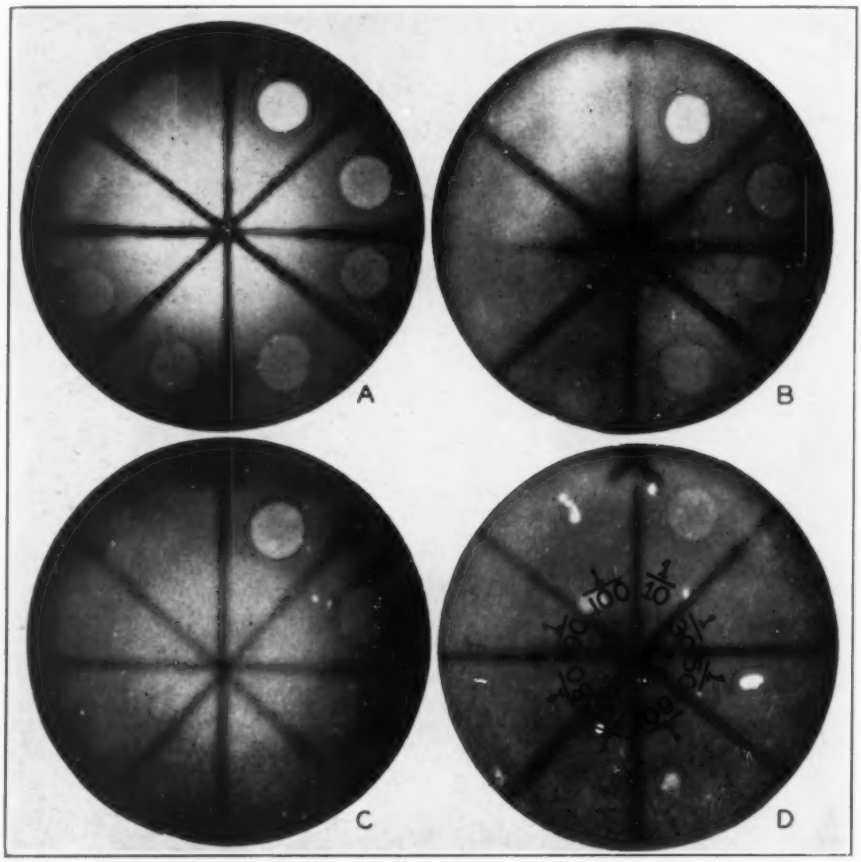


Fig. 3.—Rabbit red blood cells were added to the infusion agar and the following amounts of staphylococcus antitoxin A, 1.2 units; B, 2.0 units; C, 4.0 units; and D, 8.0 units. A drop of each dilution of staphylococcus toxin as shown in D was dropped on the surface of each plate. The amount of hemolysis decreases with an increase in the amount of antitoxin and also with a decrease in the concentration of the toxin.

antitoxin. The following dilutions of toxin in saline were used: 1:10, 1:30, 1:50, 1:60, 1:70, 1:80, 1:90, and 1:100. Immediately upon diluting the toxin a single drop through a 27 gauze needle was placed on the surface of each of the blood agar plates. The point of the needle was always held approximately the same distance from the medium. The toxin was absorbed before the plates were placed in the incubator.

Definite hemolysis was present at the site of the toxin after two hours' incubation. A record was made of the presence or absence of hemolysis at this time. Similar observations were made after the plates had been in the icebox for four and eighteen hours. Table IV shows the presence or absence

of hemolysis at these three intervals. No apparent change occurred in the plates after they had been in the icebox for seventy-two hours.

A variation in the intensity of the hemolysis occurred with the different dilutions of toxin. Hemolysis decreased with the increase of staphylococcus antitoxin (Fig. 3).

The specificity of this inhibition of hemolysis by antitoxin is shown by the fact that two hemolytic strains of streptococci produced lyses regardless of the amount of staphylococcus antitoxin present.

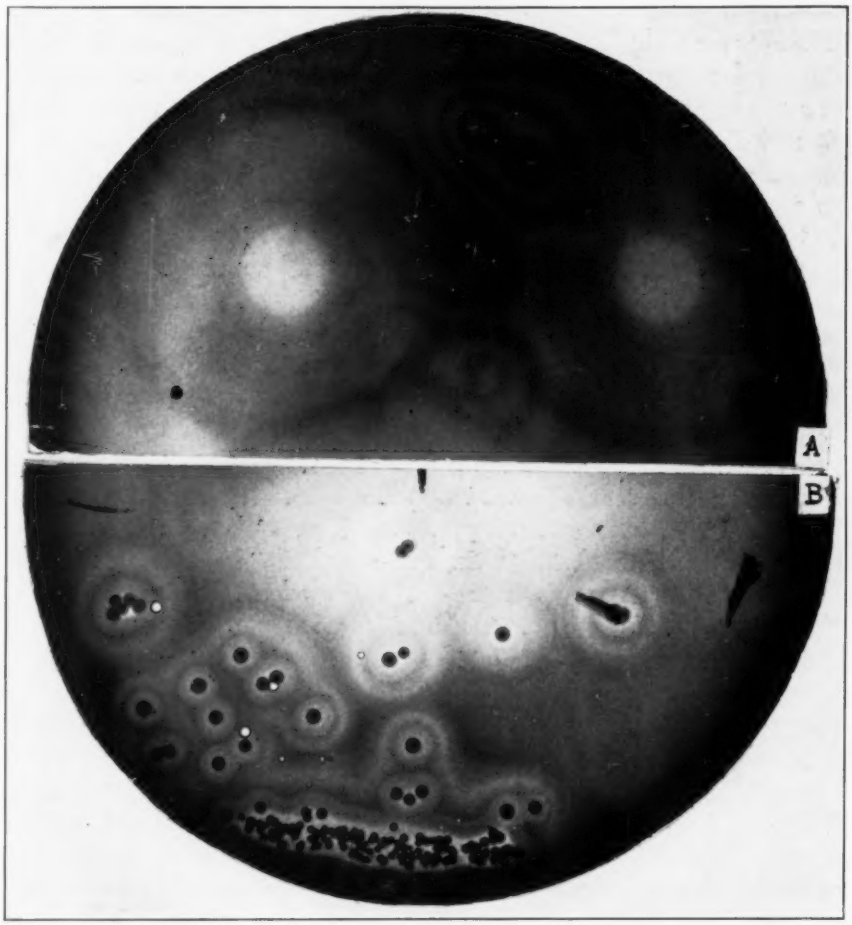


Fig. 4.—*A*, Staphylococcus toxin was dropped on the surface of a 5 per cent sheep blood agar plate in two areas. The plate was then incubated for twenty-four hours and placed in the icebox. The "zones" of hemolysis slowly developed during this period. Photographed after eleven days.

B, Zones similar to those in *A* developed around colonies of staphylococci. Photographed after four days.

Some Observations on the Process of Hemolysis Produced by Staphylococci and Staphylococcus Toxin on Blood Agar Plates.—Frequently more than a single clear zone occurs around hemolytic staphylococcus colonies growing on blood agar plates. The medium becomes clear for varying distances away from the colony. This zone is symmetrical. At the periphery of this clear

zone there often appears to be another zone darker than the adjacent medium. This latter one is also symmetrical and gradually blends into the normal color of the blood agar. Multiple zones similar to the above have been observed around colonies of staphylococcus and also in the areas where toxin has been dropped. Fig. 4 illustrates this zoning process.

The development of a clear zone and then at the periphery of it, a zone darker than the normal hemoglobin, suggested that the process may arise as a result of the diffusion of free hemoglobin outward into the medium. The multiple zones resemble the "Leisegang ring" phenomenon (Fig. 4 *A*).

Hemolysis produced by staphylococcus toxin is influenced by the temperature and the potency of the toxin as shown in the following experiments. Two groups of blood agar plates were made containing 5.0 per cent defibrinated rabbit and sheep blood. Toxin was dropped upon the surface of each. The plates were incubated for two hours at 37° C. The hemolytic areas on all plates were essentially the same size at this time. Of these, one plate each of the two types of blood was put into the icebox (5 to 15° C.) and similar plates were put into the incubator at 37° C. After twenty-four hours the plates in the icebox and incubator were sealed with putty. The cultures were examined at frequent intervals over a period of four days. At this time the hemolytic zones were slightly larger on the plates which had been kept at 37° C. This experiment was repeated with two other preparations of staphylococcus toxin, and the results were the same.

There is a wide variation in the size of the hemolytic zones produced by these three preparations of staphylococcus toxin. After the first two hours of incubation the areas of hemolysis were approximately the same size for each of the three toxins on both the rabbit and sheep blood agar. The zones varied from 1.2 to 1.5 cm. in diameter. Twenty-four hours later the areas of hemolysis produced by two of the toxin preparations were approximately the same as they were after the first two hours. The hemolytic zone produced by the third had increased 2 to 2.5 cm. in diameter. After four days the lytic areas produced by the former two preparations had increased 1.5 to 2 cm., while those areas produced by the latter measured 2.5 to 3.0 cm.

Hemolytic titrations, using rabbit red blood cells, showed that the preparation of toxin producing the largest zone of lysis on the sheep and rabbit blood agar plates contained the largest amount of hemotoxin. Regardless of this variation in potency of the toxin, it is very interesting to note that the lytic zones were all the same size after the first two hours. The lysis produced by the more potent toxin progressively increased for four days, while that produced by the weaker toxin remained essentially the same size after the first two hours.

Staphylococcus toxin when dropped upon the surface of blood agar plates apparently diffuses into the medium. The final size of the hemolytic zone apparently is influenced by whether or not all the hemotoxin unites with the cells in the immediate area. From this experiment it would appear that the hemotoxin in a potent toxin is retained in a sufficient concentration to lyze a larger number of cells as it diffuses outward into the medium. Apparently

there is no way in this experiment to determine whether the fluid constituents in all the preparations of toxin diffuse an equal distance from the point of inoculation. These observations are of interest in considering alpha and beta toxins.

That different cultures of staphylococci and toxin prepared in different media may influence the degree of lysis and the extent of diffusion appears likely.

To study the influence of the medium and the organism on the hemolysis produced by staphylococcus toxin on rabbit and sheep red blood cells, the following experiment was performed. Two liters of veal infusion were prepared, and 40 gm. proteose peptone, 10 gm. sodium chloride, and 0.6 gm. powdered agar were added. This medium was divided into two equal parts. Seven grams of sodium acetate were added to one part and 7 gm. disodium acid phosphate were added to the other. The pH was adjusted to 7.5. Five hundred cubic centimeters of the medium were put into 2 liter flasks and autoclaved. One flask containing the sodium acetate and one containing the disodium acid phosphate medium were inoculated with 1 c.c. of a twenty-four-hour staphylococcus infusion broth culture (Wood strain). The second set of flasks were inoculated with a similar amount of a broth culture of strain A-O. After forty-eight hours' incubation at 37° C. the media were centrifuged and the supernatant fluid was collected for hemolytic titrations.

The pH of the different toxin preparations was as follows:

	Strain A-O	Strain Wood
Toxin with Na_2HPO_4	pH 7.13	pH 7.105
Toxin with $NaC_2H_3O_2$	pH 6.96	pH 6.9

One per cent concentrations of washed rabbit and sheep red blood cells were used in the titrations. The tubes were kept in a water bath at 37° C. for two hours and in the icebox overnight. The following is the amount of toxin in cubic centimeters necessary to produce 50 per cent lysis of the respective red blood cells.

Strain	Toxin	Rabbit R.B.C.	Sheep R.B.C.
Wood	Na_2HPO_4	0.0008	0.13
Wood	$NaC_2H_3O_2$	0.0013	0.2
A-O	Na_2HPO_4	0.013	0.05
A-O	$NaC_2H_3O_2$	0.008	0.045

Rabbit red blood cells are more susceptible than sheep cells to staphylococcus toxin prepared in the above media and inoculated with two different strains of staphylococci. It is interesting to note that it requires less toxin from strain A-O to lyze sheep cells than it does from strain Wood. The explanation usually given for such a variation is that strain A-O contains more beta toxin than Wood strain and that the two strains have a reversed amount of the alpha toxin.

The variations in the size of the hemolytic areas on media containing blood from the rabbit and sheep apparently cannot be explained then on the basis of alpha and beta toxins. The hemolytic zones were approximately the same size on the sheep and rabbit blood agar plates when inoculated with the

toxin from either of the two strains of staphylococci used in this study. A variation in the composition of the different red blood cells and a difference in the diffusion of the toxin apparently would account for the variations observed on the blood agar plates.

To study the effect of staphylococcus toxin on hemoglobin, sheep blood was defibrinated, and 5 c.c. of the washed red blood cells were lyzed in a quantity of distilled water equal to the original volume of blood. The hemoglobin was filtered through a Berkefeld candle, and the following quantities: 1 c.c., 2 c.c., 3 c.c., 5 c.c., and 10 c.c., were added to 50 c.c. of warm infusion agar. These hemoglobin agar media were poured into Petri dishes in 10 c.c. amounts. Staphylococcus toxin was then dropped onto the surface of the plates and incubated at 37° C. for twenty-eight hours. No change different from that of the uninoculated plate had occurred at this time. The plates containing sheep red blood cells instead of sheep hemoglobin showed typical zones of hemolysis. These observations would indicate that staphylococcus toxin acts on the red blood cell in such a way that the hemoglobin escapes. Staphylococcus toxin apparently has no direct action on the diffusion or color of the hemoglobin alone.

DISCUSSION

The results of this study show that red blood cells are similarly affected by staphylococcus toxin in infusion agar and in saline suspension. The hemolysis which occurs around colonies of staphylococci, growing on the surface of blood agar plates, is influenced by the type of red blood cell used in the media. The variation in the susceptibility of red blood cells from different animals when used in the hemolytic titration of staphylococcus toxin is well known. Human blood cells in saline suspension are relatively resistant to the hemolytic action of staphylococcus toxin. Blood agar plates made with human blood do not show hemolytic zones about the colonies of staphylococci used in this experiment until approximately forty-eight hours. The same strain of organisms growing on a similar concentration of rabbit blood agar shows a wide zone of lysis after twenty-four hours. The variation in the width of the zone of hemolysis about the colonies growing on the surface of agar containing different types of blood and the delayed appearance of lysis when human blood is used are important in the differentiation of staphylococci. Colonies that do not show any hemolysis or very little after the first twenty-four hours are not generally considered pathogenic. The importance of considering the type of blood used in the differentiation of staphylococci and their hemolysins is evident.

The variation in the susceptibility of red blood cells to staphylococcus toxin may be due to differences in the structure or composition of the cells. It would appear very likely that the same variation in the red blood cell observed in titrations is also responsible for the difference observed in the hemolytic zone occurring about the colonies of staphylococci growing on the surface of agar plates. The observations made in this study indicate that naturally occurring antitoxin will not account for these variations in hemolysis.

Staphylococcus antitoxin added to blood infusion agar will inhibit the lysis produced by staphylococcus toxin dropped on the surface of the medium. The hemolysis which occurs around the colonies of staphylococcus growing on the surface of the same medium is also inhibited. Both observations illustrate the neutralization of the toxin by the antitoxin. This process of inhibition apparently is specific since two strains of hemolytic streptococci produced hemolysis on these plates.

In contrast to the absence of hemolysis in the depth of media with staphylococci, it is of interest to note that hemolytic streptococcus produced hemolysis both on the surface and in the depth of the media and that certain strains from cases of bovine mastitis are hemolytic only below the surface of the agar, while methemoglobin is produced about the surface colonies.

Zones similar to those observed in this study about staphylococcus colonies apparently were first described by Baerthlein in 1915.[5] Packalen in 1938 reviewed the theories of the formation of such processes and stated that he has adopted "as a working hypothesis the view that the areas of secondary hemolysis are the visible manifestation of invisible virus colonies deriving from staphylococcal mother colonies. The animate or inanimate nature of this virus is left open to discussion."[8]

In the present study bands or zones were observed on sheep and rabbit blood agar plates around the point where staphylococcus toxin was dropped similar to those occurring around colonies of staphylococci. The occurrence of such a process with staphylococcus filtrates would be consistent with Packalen's theory that they are the manifestation of invisible virus colonies. It would appear to me, however, that the process is more likely one associated with a diffusion of hemoglobin through the agar. A quite similar phenomenon is frequently observed in colloidal chemistry and is referred to as Leisegang rings.

The location and the irregularity of the hemolytic areas about some of the colonies, as shown by Rhodes[7] and Packalen,[8] suggest the possibility that the phenomenon observed by them is different from that described in the present paper. The processes appear so similar to me, however, that such an explanation would appear unlikely at this time. Hemolysis, whether it is around colonies of staphylococci or in the area where toxin has been dropped, suggests that in both the hemoglobin diffuses outward following the lysis of the red blood cell. The rate and the manner of diffusion of this hemoglobin may be influenced by environmental conditions. The diffusion of the toxin either from a colony or from the filtrate also may be so influenced. The location of the hemolytic areas on any blood plate would thus be influenced by such variations.

SUMMARY

The blood used in the preparation of blood agar media influences the hemolysis which occurs around staphylococcus colonies. The widest hemolytic zone about any colony of the three types of blood studied, sheep, rabbit, and man, occurs on the rabbit. The hemolytic zones develop much later on human blood than on either rabbit or sheep.

There is a variation in the size of the areas of hemolysis produced by toxin on the different types of blood agar. It is shown that this may be influenced by the potency of the toxin and by the temperature.

The type of blood used in solid media is important when one considers the phenomenon of hemolysis by staphylococci.

Staphylococcus antitoxin, when added to blood agar media, inhibits the hemolysis which occurs around the colonies. Likewise, hemolysis produced by staphylococcus toxin dropped on the surface is also inhibited by the antitoxin. This process apparently is one of neutralization of the toxin by the antitoxin and is specific, since two strains of hemolytic streptococci produced hemolysis on the plates containing staphylococcus antitoxin.

REFERENCES

1. Velde, van de: Etude sur le mechanism de la virulence der staphylocoque pyogene, La Cellule **10**: 403, 1894.
2. Kraus, R., and Clairmont, P.: Ueber Hemolysin und antihemolysine, Wien. klin. Wchnschr. p. 49, 1900.
3. Neisser, M., and Wechsberg, F.: Ueber eine neue einfache Methode zur Beobachtung von Schadigungen lebender zellen und Organismen, München. med. Wchnschr., p. 37, 1900.
4. Gross, H.: Die Fermente und Giftstoffe der Staphylokokken, Ergebn. d. Hyg. Immunitätsforsch. u. exper. Therap. **13**: 516, 1932.
5. Baerthlein, K.: Ueber Blutveranderungen durch Bakterien, Zentralbl. f. Bakt. (I, Orig.) **74**: 201, 1914.
6. Naidu, P. M. N.: Zur hemolyse der Staphylokokken in Schafblutazarplatten, Zentralbl. f. Bakt. **47**: 132, 1934.
7. Rhodes, Bernice A.: Satellite Hemolytic Zones in Blood Agar Staphylococcus Cultures, J. Infect. Dis. **62**: 124, 1938.
8. Packalen, T.: A Peculiar Type of Hemolysis Produced by Staphylococcus Aureus, Acta path. et microbiol. Scandinav. **15**: 117, 1938.
9. Bigger, J. W., Boland, C. R., and O'Meara, R. A. Q.: A New Method of Preparing Staphylococcal Hemolysin, J. Path. & Bact. **30**: 271, 1927.

ESTROGENIC AND CHORIONIC GONADOTROPIC HORMONE IN NORMAL PREGNANCY AND IN TOXEMIA OF PREGNANCY

SAMUEL L. SIEGLER, M.D., F.A.C.S., BROOKLYN, N. Y.

THE presence of estrogenic and chorionic gonadotropic hormone in pregnancy has been demonstrated by Zondek,[1] Smith,[2] Evans,[3] Frank,[4] Cohen and others.[5] They have shown that the amounts of these hormones differ in different individuals, and at different periods of gestation. Smith and Smith[6] made a careful comparison of a twenty-four-hour elimination of gonadotropic and estrogenic hormones of normal pregnancy and of pregnancy complicated by disease (kidney disease, pre-eclampsia, and eclampsia) and concluded that there is an excessive amount of the gonadotropic hormone and a subnormal amount of the estrogenic hormone in toxemias of late pregnancy and eclampsia. They also showed that the excessive amount of the gonadotropic hormone had probably been present for some time before the appearance of symptoms in the cases of late toxemias. High levels of gonadotropic hormone during the sixth and seventh months in apparently normal women may indicate that toxemia may develop. Heim,[7] however, observed a very high excretion of both gonadotropic and estrogenic hormones in urines of toxemia of pregnancy, while Bichenbach and Fromme[8] could find no noteworthy increase in follicular hormone in cases of eclampsia over the amount found in normal pregnant women.

The purpose of this report is to record the quantitative determination of the total estrogenic and chorionic gonadotropic hormones in the cases of 5 normal pregnancies, and in one case of toxemia of pregnancy, which toxemia was alleviated by the administration of large doses of estradiol benzoate.

METHODS

The Bergen-Smelzer[9] modification of the Kurzrok-Rattner method was used for the extraction of the estrogenic hormone from twenty-four-hour specimens of urine, collected at weekly intervals. From 10 to 20 spayed rats were used in the estimation, and results were expressed in rat units. Only those rats which showed an estrous effect in vaginal smears were considered positive. The estrous effect was recognized by an absence of leucocytes and the presence of cornified epithelial cells. In several instances the Frank-Salmon method for the determination of blood estrin was used. In the assaying of the gonadotropic hormone, the Aschheim-Zondek method was employed for both urine and blood determinations. Three immature female mice were used for each dose level over a range of at least four different levels.

CASE REPORT

Mrs. M. K., aged 29 years, para i, gravida ii, was married five years. First pregnancy ended in an abortion at five months; the cause unknown. Menses started at 14 years, recurred every twenty-eight days, with a duration of four days. No dysmenorrhea. Last menstrual

period was Dec. 4, 1937. Expected date of confinement was Sept. 11, 1938. Mensuration was normal. Twins were delivered on Sept. 24, 1938. Male weighed 8 pounds 3 ounces; female, 8 pounds 5 ounces; total weight 16½ pounds.

TABLE I

DATE	WT.	B.P.	URINE ALB.	HORMONE DETERMINATION		SYMPTOMS AND SIGNS OF TOXEMIA
				EST.*	G. H.†	
5/29	166½	120/ 70	Negative	3,700	3,000	None
6/23	172	110/ 80	Negative	5,600	4,000	None
7/28	178½	140/ 78	1 plus	12,200	10,000	Edema of ankles
8/25	184	148/ 90	2 plus	23,500	18,000	Edema of ankles, face, hands
9/12	186	170/100	4 plus	31,700	49,000	Severe headache; epigastric distress and nausea. Polyhydramnios and edema as above
‡Estradiol benzoate,* 1,000,000 I.U. in divided doses given in following 10 days						
9/22	186	126/ 86	Slight trace	52,000	12,000	Edema negligible Headaches subsided after second injection. Epigastric distress disappeared
4 days post-partum		120/ 80	Negative	180	0	

*Est. = Estrogen.

†G. H. = Gonadotropic hormone.

‡The estradiol benzoate used was that supplied by the Italian Drug Importing Co. under the trade name of Ectofolliculina, and standardized by comparing its estrogenic activity with that of the International Reference Samples distributed by the League of Nations, one unit representing the specific estrous producing activity of 1/10 gamma (1/10,000 mg.) of the crystalline estrone standard assayed according to the procedure laid down by the Hampstead Institute in 1935.

RESULTS

Fig. 1 shows an average gradual increase in the total estrogenic hormone eliminated in the 5 normal cases of pregnancy, from 3500 R. U. per liter on the thirtieth day following the first missed menstrual period, reaching a peak at term of 82,000 R. U., after which there was an abrupt decline of the concentration of the total estrogenic hormone to 2200 R. U. on the first day post partum to 200 R. U. on the fourth day post partum. The lowest amount excreted on the thirtieth day was 1800 R. U., and the highest amount on that day was 4800 R. U. The lowest concentration at term was 70,000 R. U., and the highest was 100,000 R. U. A graph of composite results is recorded.

Conversely, in the determination of the chorionic gonadotropic hormone, one finds a sudden increase from 7500 M. U. on the fourteenth day after the first missed menstrual period, to its peak of 140,000 M. U. on about the thirtieth day. Thereafter there is an abrupt decline in the concentration of gonadotropic hormone, with a level below 10,000 M. U. per liter after the sixtieth day to an average of about 4500 M. U. to the termination of pregnancy, becoming negative on the fourth day post partum. The lowest concentration in the first two weeks following the first missed menstrual period was 4500 M. U., and the highest amount was 15,000 M. U. The lowest amount at the peak was 96,000 M. U., and the highest amount was 210,000 M. U.

In the several instances where the serum had been used in the assaying of the total estrogenic and gonadotropic hormones, the amounts estimated coincided with those found in the urine. The graph (Fig. 2) of hormonal determination

in toxemia of pregnancy indicates a rise in the amount of gonadotropic hormone in the fifth and sixth months. An increase in blood pressure, the presence of albuminuria and edema in the following month with a higher level of gonadotropic hormone concentration, and a subnormal amount of total estrogenic hormone followed. These higher levels of gonadotropic hormone and lower levels of total estrogenic hormone persisted with the severe toxemic symptoms until the administration of estradiol benzoate. Following this treatment, the level of gonadotropic hormone fell to almost normal, the level of the total estrogenic hormone rose in amount, and the toxemic symptoms subsided.

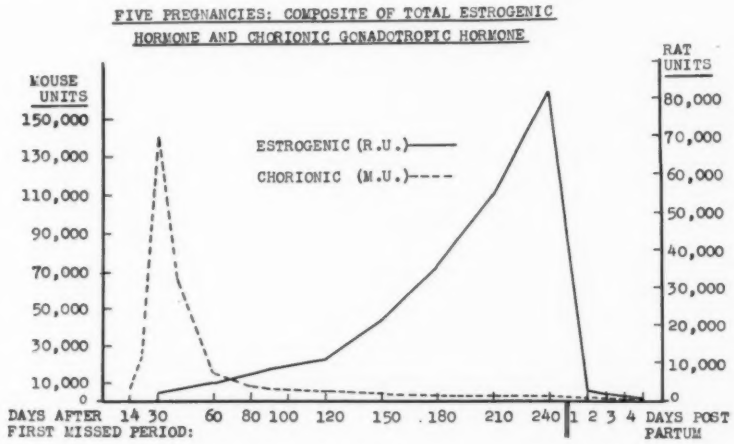


Fig. 1.

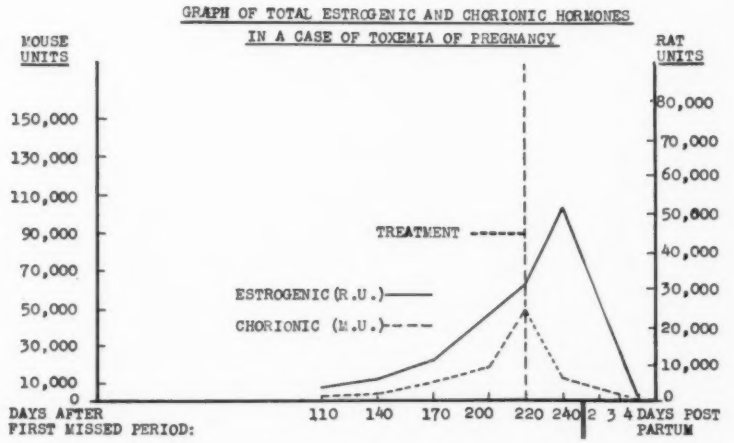


Fig. 2.

DISCUSSION

In evaluating the data herein presented, one should be hesitant in stating, as pointed out by Evans and others,[3] that the sudden early increase in the gonadotropic hormone yielded by the urine or blood is indicative of toxicosis. As in his series, the patients with the highest concentration of gonadotropic hormone and the lowest concentration of estrogenic hormone were comparatively free from the nausea and vomiting of early pregnancy.

Heim[10] in 1936 found markedly increased values of anterior pituitary-like substance in 8 out of 9 cases of placental separation. Tell and Zondek, cited by Heim, were able to produce placental separation in pregnant animals by the administration of gonadotropic hormone in large quantities. Shute[11] showed that there was an increase of gonadotropic hormone and total estrogenic hormone in the blood in cases of toxemia associated with abruptio placentae which were benefited by the use of wheat germ oil.

In the toxemias it is a question whether the hormones produced are of pituitary or placental origin, and whether they are the cause or the effect of the toxemia. Many theories have been expressed as to which is the motivating factor. Smith and Smith[12] contend that the overproduction of gonadotropic hormone by the placenta is probably a related factor, and possibly causal in the etiology of toxemia of late pregnancy and eclampsia. In their[13] latest contribution to the subject they postulated that the vascular phenomena which are responsible for endometrial flow and pre-eclampsia may be brought about by a toxic concentration of nonestrogenic breakdown products as a result of progestin deficient metabolism of the estrogens. Cushing,[14] and Anselmino and Hoffman[15] contend that the pituitary dysfunction associated with toxemia and eclampsia involves the pressor and antidiuretic factor of the posterior lobe and as such may be considered as the cause of the excessive prolan in the blood, serum, and urine. Clinically one recognizes lesions in the placenta, liver, and kidneys, and not infrequently, in the brain, and retinal changes in cases of toxemia of pre-eclampsia and eclampsia. Is there not a possibility that the deviation from the normal of the gonadotropic and total estrogenic hormones found in these cases, in both the urine and blood, might be the result of disorders in the lesions above described, instead of the cause? At best, the problem still leaves a fertile field for investigation.

CONCLUSIONS

1. Quantitative determinations of estrogenic and chorionic gonadotropic hormones in the cases of 5 normal pregnancies have been recorded. There is a gradual increase of the total estrogenic hormone which reaches its peak at or near the termination of pregnancy. Secondly, there is a gradual increase in the chorionic gonadotropic hormone, reaching its peak at about the thirtieth day following the first missed menstrual period, after which there is an abrupt decline between the thirtieth and fortieth days, remaining well below 10,000 M. U. to the termination of pregnancy.

2. In a case of toxemia of pregnancy there was an excessive amount above the average normal of anterior pituitary-like substance at about the sixth or seventh month of pregnancy, with a decrease of the total estrogen to term.

3. The efficacy of estrogenic substance in the treatment of a case of toxemia of pregnancy is demonstrated.

This work was aided by a grant from Dr. Sant Piracci, of the Italian Drug Importing Co.

The author is indebted to the Misses Antonina P. Chiaramonte and Josephine M. Bearss for their assistance in the experimental work in the Endocrine Laboratory, Unity Hospital in Brooklyn.

REFERENCES

1. Zondek, B.: Die Schwangerschaft aus dem Harn durch Nachweis des Hypophysenvorderlappens Hormons, Klin. Wchnschr. **7**: 1404, 1938.
2. Smith, M. G.: A Study of the Ovarian Follicular Hormone in the Blood of the Pregnant Woman, Bull. Johns Hopkins Hosp. **41**: 62, 1937.
3. Evans, H. M., Kohls, C. L., and Wonders, D. H.: Gonadotropic Hormone in the Blood and Urine of Early Pregnancy, J. A. M. A. **108**: 287, 1937.
4. Frank, R. T.: Sex-Endocrine Factors in Blood and Urine in Health and Disease, J. A. M. A. **104**: 1991, 1935.
5. Cohen, S. L., Marrian, G. F., and Watson, M.: Excretion of Estrin During Pregnancy, Lancet **1**: 674, 1935.
6. Smith, G. V., and Smith, O. W.: Excessive Gonadotropic Stimulating Hormone and Subnormal Amounts of Oestrin in the Toxemias of Late Pregnancy, Am. J. Physiol. **107**: 128, 1934.
7. Heim, K.: Umvertung der quantitativen Hormonanalyse bei Hyperprolanurie, Klin. Wchnschr. **14**: 496, 1935.
8. Bichenbach, W., and Fromme, H.: Uber den Follikelhormongehalt des Blutes Eklamptischer, Klin. Wchnschr. **14**: 166, 1935.
9. Kurzrok, R.: The Endocrines in Obstetrics and Gynecology, Baltimore, 1937, p. 451, Williams & Wilkins Co.
10. Heim, K.: Quantitative Untersuchungen zum Prolan-Problem, Arch. f. Gynäk. **161**: 293, 1936.
11. Shute, Evan: The Endocrine Background of the Toxemias of Late Pregnancy, Surg., Gynec. & Obst. **65**: 480, 1937.
12. Smith, G. V., and Smith, O. W.: Evidence for the Placental Origin of the Excessive Prolan of Late Pregnancy Toxemia and Eclampsia, Surg., Gynec. & Obst. **61**: 175, 1935.
13. Smith, G. V., and Smith, O. W.: Observations Concerning the Metabolism of Estrogens in Women, Am. J. Obst. & Gynec. **36**: 769, 1938.
14. Cushing, H.: Hyperactivation of the Neurohypophysis as the Biological Basis of Eclampsia and Other Hypertensive States, Am. J. Path. **10**: 145, 1934.
15. Anselmino, K., Hoffman, F., and Kennedy, W. P.: The Relation of Hyperfunction of the Posterior Lobe of the Hypophysis to Eclampsia and Nephropathy of Pregnancy, Edinburgh M. J. **39**: 376, 1932.

SULFAPYRIDINE IN BLOOD OF GUINEA PIGS AFTER ORAL ADMINISTRATION*

ARTHUR RILEY ARMSTRONG, B.S., M.D., AND DAVID ROBERTSON MUIRHEAD, M.T.
ONTARIO, CANADA

IN CONNECTION with an investigation of the therapeutic value of sulfapyridine against tuberculosis in guinea pigs, we wished to determine the dosage regime which would maintain a concentration of the free drug in the blood at or above 7 mg. per cent. This level was selected arbitrarily because of the efficacy of sulfapyridine and other similar chemotherapeutic agents at this concentration against organisms other than the tubercle bacillus. Preliminary experiments with guinea pigs were, therefore, made to determine the blood levels resulting from single and repeated doses of sulfapyridine. The following data, obtained from over 200 guinea pigs, are typical of our findings.

The animals used in these tests ranged in age from 8 months to 1½ years, and in weight from 500 to 800 gm. During the experiments no change in their

*From the Department of Pathology, Mountain Sanatorium.

Received for publication, May 22, 1939.

The sulfapyridine used in these experiments was kindly supplied by Poulenc Freres of Montreal under the trade name "Dagenan."

TABLE I

BLOOD SULFAPYRIDINE AFTER 50 MG. DOSES GIVEN EVERY FOUR HOURS

TIME OF OBSERVATION IN HOURS AFTER FIRST DOSE	BLOOD SULFAPYRIDINE MG. PER CENT			
	1 (LOWEST VALUE)	2	3	4 (HIGHEST VALUE)
1	0.4	0.4	1.4	2.6
2	0.4	3.0	3.0	3.4
3	2.6	2.8	2.8	3.2
4	0.8	1.0	1.2	1.2
5	2.8	2.8	3.2	3.6
6	6.4	6.8	7.6	8.0
7	2.8	4.0	4.4	4.4
8	1.4	1.6	2.2	2.4
9	2.6	3.6	4.2	4.4
10	3.6	4.8	4.8	5.8
11	2.4	2.6	3.2	3.6
12	1.2	1.6	1.6	2.8

regular diet was made. Four animals were sacrificed to establish the sulfapyridine level at any given time. In most cases the concentration of drug in the four bloods agreed remarkably well, as can be seen in the tables.

Three dosage levels were studied. The individual doses used were 50 mg., 100 mg., and 200 mg., respectively, in the three series of experiments. The sulfapyridine was administered as a suspension,* stabilized by the addition of agar-agar. This suspension was sufficiently fine to pass readily through a No. 21 gauge hypodermic needle and a No. 6 French gauge ureteral catheter, employed as stomach tube. The amount of suspension given was always 2 c.c. (Approximately one animal can be fed per minute in this fashion.)

The method employed for estimating blood sulfapyridine was practically identical with Marshall's method[1] for sulfanilamide, the standard, of course, being sulfapyridine instead of sulfanilamide. The color developed was read against the standard after thirty minutes or more.

It is customary to give drugs on the basis of body weight, but our data indicate that if the animals are of about the same size and age this may be an unnecessary refinement. Although doses of fixed quantity were used, when each animal was weighed at the time of killing no correlation could be found between weight and drug concentration in the blood. The blood level is presumably controlled by the relative rates of absorption, conjugation, and excretion. In guinea pigs the effect of weight appears to be insignificant compared to the variations in the rates of these three processes.

A 50 mg. dose, repeated every four hours for three doses, generally failed to produce the desired blood concentration of 7 mg. per cent (see Table I). On the other hand, repeated 200 mg. doses gave values much higher than desired (see Table III). However, 100 mg. given every four hours (see Table II) produced, after three doses, approximately the blood levels desired, and

*The details of preparing such a suspension are as follows: If the sulfapyridine supplied is already in a very finely divided state, 4 gm. is shaken with 50 ml. water in a stoppered 100 ml. cylinder. Twenty cubic centimeters of 1 per cent hot agar solution is added and mixed. The volume is made to 80 c.c., and the suspension is thoroughly mixed. It is cooled under the tap until the material tends to remain suspended for some time. If the chemical is in too coarse a state, the 4 gm. are dissolved in 45 c.c. hot normal hydrochloric acid. Two drops 1 per cent phenolphthalein are added, and *while still hot* the solution is neutralized with 40 per cent sodium hydroxide, adding 0.5 c.c. portions at a time, with complete mixing. Then the agar is added and the volume made to 80 c.c. as before.

TABLE II

BLOOD SULFAPYRIDINE AFTER 100 MG. DOSES GIVEN EVERY FOUR HOURS

TIME OF OBSERVATION IN HOURS AFTER FIRST DOSE	BLOOD SULFAPYRIDINE MG. PER CENT			
	1 (LOWEST VALUE)	2	3	4 (HIGHEST VALUE)
1	1.6	2.4	4.0	6.6
2	0.4	2.0	5.2	5.2
3	4.2	4.4	4.8	4.8
4	3.0	3.6	3.6	4.8
5	7.4	7.6	8.0	8.2
6	6.8	7.2	7.6	10.0
7	4.2	4.8	4.8	5.2
8	3.6	4.0	4.2	4.4
9	8.8	9.2	9.2	11.2
10	10.8	11.4	11.6	12.0
11	6.0	6.0	6.8	10.0
12	6.4	7.2	7.2	8.0
14	14.4	16.0	17.6	20.0
16	11.0	12.8	12.8	16.8
18	14.4	15.2	16.8	20.0
20	11.6	11.6	13.2	14.8
22	15.4	16.0	16.4	22.4
24	13.6	13.6	14.8	18.0
26	13.4	14.0	15.2	17.8
28	11.0	11.2	11.8	15.4
30	8.4	8.8	11.5	14.9
32	11.2	11.6	12.8	15.2
36	7.2	8.0	9.6	12.8
40	9.2	9.6	10.0	11.2
44	8.8	9.2	12.0	13.6
48	11.2	12.4	12.8	17.2
72	8.8	9.2	10.4	12.0
96	10.8	11.2	12.0	12.0
120	7.2	12.0	13.2	14.8
144	14.0	17.6	18.8	18.8
164	13.2	13.6	15.2	17.6

TABLE III

BLOOD SULFAPYRIDINE AFTER 200 MG. DOSES GIVEN EVERY FOUR HOURS

TIME OF OBSERVATION IN HOURS AFTER FIRST DOSE	BLOOD SULFAPYRIDINE MG. PER CENT			
	1 (LOWEST VALUE)	2	3	4 (HIGHEST VALUE)
1	1.0	3.2	3.2	3.2
2	6.0	9.2	10.0	10.8
3	8.6	9.2	10.6	10.8
4	8.8	9.2	9.6	10.0
8	17.0	17.3	20.4	21.0
12	13.6	14.0	18.8	24.5

these were maintained throughout the experiment (one hundred and sixty-four hours).

The animals were observed for signs of toxicity, but all remained apparently quite well, even those that received 41 doses of 100 mg. In spite of extreme gentleness in passing the tube at the feedings, nearly all stomachs examined postmortem presented slight abrasions, and in one case the stomach was found to have been punctured by the catheter.

The authors wish to thank Dr. W. S. Stanbury, the Director of Laboratories, for making it possible to conduct this investigation and for his kindly interest throughout; and also H. Norris, G. Clark, and H. Saunders, for their assistance in handling the animals.

REFERENCE

1. Marshall, E. K., Jr.: Determination of Sulfanilamide in Blood and Urine, J. Biol. Chem. **122**: 263, 1937.

LABORATORY METHODS

A RAPID METHOD FOR THE DETERMINATION OF WATER IN BIOLOGICAL MATERIAL*

ALEXANDER S. CHAIKELIS, PH.D., NEW YORK, N. Y.

A RAPID and reliable procedure for routine determinations of water in biological fluids of small quantity is herewith presented. It has been frequently pointed out that the ordinary method of water determination by drying to constant weight, at a temperature of 105° to 110° C., does not exclude the important sources of error in samples containing protein material: namely, adsorption, occlusion, and chemical reaction. Occlusion, as a source of error, is most frequently encountered in the determination of water in blood. A solid pellicle of organic material forms at the bottom of the glass-weighing bottles employed in the drying process. This formation resists any further attempt to dehydrate the sample except where the solid mass is broken up with the subsequent unavoidable loss of some of the sample. Shackell[1] found that freezing the sample, followed by desiccation through the use of the so-called "Geryk" type pump, eliminated all the unfavorable effects of high temperature drying.

The method advocated in this paper is an application and refinement of the basic idea of using alcohol-xylene mixture as presented by Dolch and Poechmueller.[2] It is applicable to the determination of water in samples of biological material, the quantity of which is sufficiently small to permit frequent sampling for analysis.

Inasmuch as this method of analysis is based on the relation of temperature to the relative solubility of water in a mixture of ethyl alcohol and xylene, an alcohol calibration curve (or table) is of primary necessity. The temperature at which various proportions of water are miscible in an ethyl alcohol-xylene mixture of constant proportion is directly related to the amount of water present. The quantity of water, by weight, bears a linear relationship to the temperature. The calibration curve is a straight line.

The Calibration Curve.—In the process of obtaining the calibration curve, the employment of absolute ethyl alcohol was found to be inexpedient for samples as small as 1 ml.

The alcohol used throughout this investigation was 190 proof ethyl alcohol (93 per cent alcohol by weight). To obtain the various points needed to construct the calibration curve, the following procedure was employed:

*From the Department of Physiology, College of Physicians and Surgeons, Columbia University, New York.

Received for publication, November 3, 1938.

1. Into each one of 6 weighed and glass-stoppered Erlenmeyer flasks of 125 ml. capacity 25 ml. of ethyl alcohol was pipetted; then the flasks were weighed.

2. To each one of the above flasks there was now added a measured quantity of distilled water at room temperature, so that each flask contained, respectively, the following amount of water: 0.5 ml., 0.6 ml., 0.7 ml., 0.8 ml., 0.9 ml., 1.0 ml. Once more the flasks were weighed to determine the weight of water added.

3. Twenty milliliters of xylene (water and acid free, and stored over sodium) were pipetted into each one of 6 pyrex test tubes (200 by 25 mm.). Then a 20 ml. aliquot of each one of the ethyl alcohol-water mixtures was pipetted into its corresponding test tube. The resulting xylene-ethyl alcohol-water mixture was now agitated to obtain homogeneity of appearance.

4. Each one of the test tubes was now placed in a special temperature recording chamber (description of which follows in another section of this paper), and the temperature of the "critical point" was determined. The "critical point" is that temperature at which the turbidity of the mixture is uniform throughout the mixture.

TABLE I

RELATION OF THE WEIGHT OF WATER TO THE TEMPERATURE OF THE "CRITICAL POINT" IN A XYLENE-ETHYL ALCOHOL-WATER MIXTURE, CONTAINING 20 C.C. XYLENE AND 20 C.C. ALCOHOL AND WATER

AMOUNT OF DIST. WATER DELIVERED	WEIGHT OF WATER	TEMPERATURE "CRITICAL POINT"
c.c.	*Mg.*	*C.*
0.5	488	−18.5
	493	18.3
	498	18.1
0.6	576	15.1
	591	14.5
0.7	694	10.5
	702	10.2
0.8	789	6.8
	793	6.6
0.9	885	3.1
	897	2.6
1.0	973	0.3
	1010	+1.8

With the exception of the first ethyl alcohol-water mixture, which was performed in triplicate, every one of the other mixtures was carried out in duplicate. The weight of the water, to the nearest milligram, was then plotted as the ordinate and the "critical point" temperature as the abscissa. Table I gives the values for each one of the determinations of the measured quantities of water added to the ethyl alcohol. From the calibration curve (Fig. 2) the conversion table as indicated in Table II was obtained.

Temperature Recording Apparatus.—A brief description of the special recording apparatus (Fig. 1) follows: It consists of a cooling chamber constructed of wood, lined with galvanized sheet metal, and insulated with com-

TABLE II

RELATION OF WEIGHT OF WATER IN MG. TO THE TEMPERATURE OF THE "CRITICAL POINT" IN A XYLENE-ETHYL ALCOHOL-WATER MIXTURE CONVERTED FROM FIG. 2.

TEMPERATURE "CRITICAL POINT"	WEIGHT OF WATER	TEMPERATURE "CRITICAL POINT"	WEIGHT OF WATER
C.	*Mg.*	*C.*	*Mg.*
−1.0	939	−4.0	862
1.5	926	4.1	859
2.0	913	4.2	857
2.5	901	4.3	854
3.0	888	4.4	851
3.5	875	4.5	849
4.0	862	4.6	846
4.5	849	4.7	844
5.0	836	4.8	841
5.5	823	4.9	838
6.0	810	5.0	836

TABLE III

EFFECT OF TIME ON THE QUANTITY OF WATER EXTRACTED BY ALCOHOL (93 PER CENT BY WEIGHT) FROM A 1 ML. SAMPLE OF RABBIT'S BLOOD, AT ROOM TEMPERATURE

WEIGHT OF SAMPLE	EXTRACTION TIME	PERCENTAGE OF WATER BY WT.	REMARKS
Mg.	*Min.*		
1.0371	10	83.6	
1.0370	20	84.1	
1.0391	30	84.7	Minimum
1.0382	60	84.71	Maximum
1.0437	1,440	84.73	

position cork. The front part of the chamber has a simple arrangement fashioned out of the sheet metal to enable the insertion of the test tube containing the mixture to be cooled. This tube is cooled very efficiently with crushed "dry ice" which can produce the extremely low temperatures that are essential for this method.

The light source consists of a concentrated filament bulb (50 watt, 110 volt) enclosed in a compartment. The light beam is concentrated by a small reflector in the compartment, and after passing through a condenser it is directed into the test tube through an aperture in the bottom of the cooling chamber. The cooling chamber rests on the light-source box by means of supports. Thus the temperature chamber and the light-source compartment form a compact unit that is easily transported.

The thermometer is alcohol-filled. The graduations begin 5 inches above the bulb to enable easy reading; the unit graduations are 1 cm. apart. Each degree on the thermometer is subdivided into tenths. The degree marks extend from −20° C. to +20° C.

Procedure for the Determination of Water in 1 ml. Sample of Blood.—The following procedure was adopted for the determination of water in a 1 ml. sample of blood. With this method an accuracy of 0.003 is obtainable.

1. Pipette approximately 1 ml. of blood into a weighed Erlenmeyer flask of 125 ml. capacity, containing 25 ml. ethyl alcohol. Stopper the flask and weigh it. Allow the flask to stand for thirty to sixty minutes (see Table III) with an occasional thorough agitation of its contents.

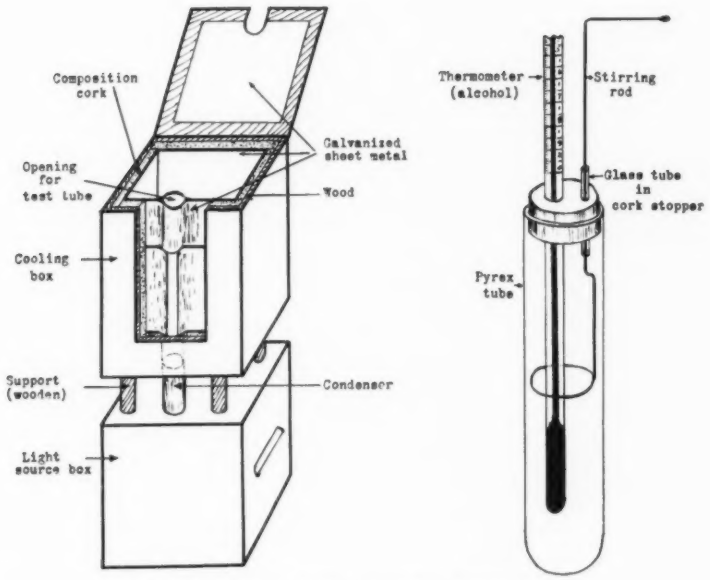


Fig. 1.—Temperature recording apparatus.

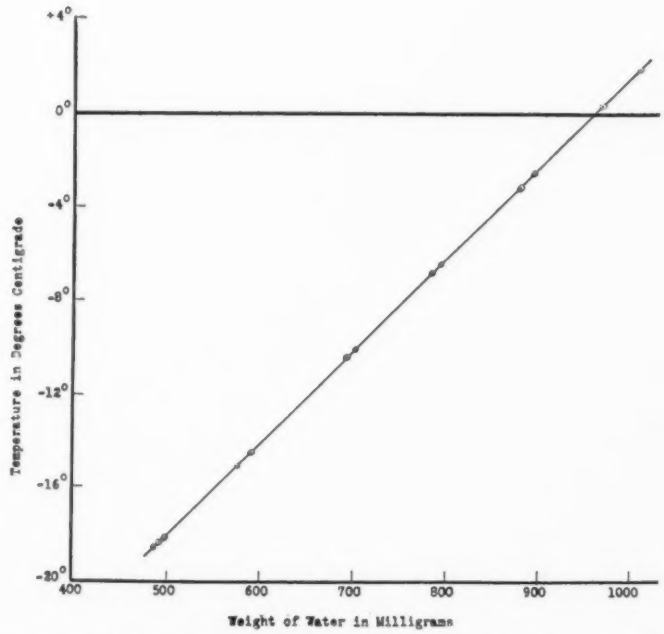


Fig. 2.—Calibration curve for the alcohol-xylene method of determination of water content. The alcohol was ethyl (93 per cent by weight).

2. Filter contents of the flask through moisture-free paper into a dry test tube.

3. Take a 20 ml. aliquot and mix it with 20 ml. dry xylene in the pyrex tube.

4. Place the tube in the cooling apparatus and determine the temperature of the "critical point" of the mixture.

5. By using the conversion table, find the amount of water in milligrams that corresponds to the temperature noted.

6. This value multiplied by 100 and divided by the weight of the sample is the percentage of water by weight in the sample.

Critical Examination of the Method.—The proposed method of water determination was tested on ox blood, obtained from the abattoir, by doing three short series of five observations each. The results obtained are tabulated in Table IV. Though too few in number to permit prediction, these results, nevertheless, gave good indications of being adequate when compared with the drying method. The procedure of the drying method was to desiccate the sample at an oven temperature of 40° C. for seventy-two hours, and follow that by a subsequent heating at a temperature of 105° C. for three hours.

TABLE IV

COMPARISON BETWEEN THE STANDARD DRYING METHOD OF DETERMINING WATER CONTENT AND THE PROPOSED EXTRACTION METHOD. OX BLOOD WAS USED AS THE EXPERIMENTAL SUBSTANCE

CONDITION OF SAMPLE	WEIGHT OF SAMPLE MEANS* (IN GM.)	WEIGHT OF WATER DRYING (IN GM.)	WEIGHT OF WATER EXTRACTION (IN GM.)	PER CENT DIFFERENCE
Defibrinated ox blood	1.031	0.809	0.845	3.5
Ox blood plus 0.5 ml. water	1.501	1.281	1.298	1.1
Ox blood plus 1 ml. water	2.029	1.793	1.849	2.8
Average	1.520	1.294	1.331	2.4

*The *means* indicated above represent the means of five observations.

The method was more rigorously tested by comparing it with the drying procedure on a long series of 62 observations carried out on human diabetic blood. The results of this phase of the investigation appear in Table V. Comparison of the two means by the method of dividing the *observed difference between the two means* by the *probable error of the difference between the two means* gave evidence of the fact that this extraction method yields results that are consistently higher in value than those obtained by the drying method. In Table IV we see that the average per cent difference between the extraction and the drying method is only 2.4 per cent even with so short a series of observations. In Table V we see that the difference is 1.6 per cent, being higher by that amount than the mean value for the observations on the drying method.

That the higher values yielded by the extraction method were due to a more thorough removal of water than to the possible effect of alcohol-soluble material extracted from the blood was proved by the following experiment: The procedure for blood analysis was carried out to the point just beyond the

filtration step. The 20 ml. aliquot of the filtrate was evaporated to dryness. To the residue was added 20 ml. of a mixture of absolute ethyl alcohol (99.8 per cent) and distilled water. The quantity of water in the mixture was equal in volume to 1.50 ml. The temperature of the "critical point" of this mixture was identical with the one determined on the mixture of ethyl alcohol and water alone. Thus alcohol-soluble material was not responsible for the consistently high values obtained through the use of this extraction method.

TABLE V

COMPARISON BETWEEN THE STANDARD DRYING METHOD OF DETERMINING WATER CONTENT AND THE PROPOSED EXTRACTION METHOD CARRIED OUT ON A LONG SERIES OF OBSERVATIONS (62) ON HUMAN DIABETIC BLOOD

PER CENT WATER DRYING METHOD			PER CENT WATER EXTRACTION METHOD			DIFFERENCE BETWEEN THE MEANS	PROBABLE ERROR OF DIFFERENCE BETWEEN MEANS
MEAN	E*	EM†	MEAN	E*	EM†		
79.0	1.94	0.25	80.6	1.78	0.23	1.6	0.229330

*The mean deviation rather than the standard deviation was used as suggested by Scott.[3]

†The mean deviation of the mean (ϵ_M) was calculated from: $\epsilon_M = \frac{\epsilon}{\sqrt{N}}$

The probable error of the difference between the two means was determined by using the formula given by Chaddock[4]:

$$\text{P. E.}_{(M_1 - M_2)} = 0.6745 \sqrt{(\epsilon_{M1})^2 + (\epsilon_{M2})^2}$$

The problem of duration, from the point of view of time, of extraction required to give comparable values in duplicates of the same sample was next investigated. Weighed samples of rabbit blood were allowed to remain in contact with ethyl alcohol at room temperature (22° C.) for varying periods of time, ranging from ten minutes to twenty-four hours. The percentage of water by weight was then calculated. The results are summarized in Table III. It can be seen that no significant advantage can be gained by leaving the sample to extract longer than the maximum time of sixty minutes; therefore any interval between the minimum time (thirty minutes) and the maximum time (sixty minutes) is sufficiently effective. The *total analysis time* by the extraction method was found to be one and one-half hours, as compared with seventy-five hours by the drying method.

This extraction method is at present being applied to the study of water content in milk (human and cow), cheese, gasoline, tissues (muscle, nerve, gland, etc.), paper, cotton, and alcoholic beverages, where preliminary tests have shown the efficacy of this rapid and reliable method.

SUMMARY

1. A rapid method for the determination of water in biological material, embodying alcohol extraction and xylene-alcohol-water miscibility principle, with special emphasis on the determination of water in whole blood in samples of 1 ml., has been perfected.

2. This method subjected to the determination of water in human diabetic blood in a series of 62 observations has been found to give consistently good results, requiring but a maximum analytical period of one and one-half hours as contrasted with the tedious seventy-five hours' determination period employing the standard drying method.

3. This method gives results that are but approximately 2 per cent higher than those carried out with the standard drying method.

4. The sensitivity of the method was found to the extent of 3 parts in 1,000.

5. Description of an inexpensive, but extremely efficient, cooling apparatus, wherein temperatures better than −30° C. are obtainable, is given.

The author desires to express his sincere thanks to Dr. Horatio B. Williams and Dr. Ernest L. Scott, of he Department of Physiology, College of Physicians and Surgeons, Columbia University, for their stimulating interest and valued advice in organizing the presentation of this investigation; and to Dr. William Ward Browne, of the Department of Biology, The College of the City of New York, for his valued suggestions and for making it possible to obtain the "dry ice."

REFERENCES

1. Shackell, L. F.: An Improved Method of Desiccation With Some Applications to Biological Problems, Am. J. Physiol. **24**: 325, 1909.
2. Dolch, M., and Poechmueller, E.: Eine exakte Methode zur Bestimmung von Wasser, Ztschr. f. physiol. Chem. **195**: 28, 1931.
3. Scott, E. L.: What Constitutes an Adequate Series of Physiological Observations? J. Biol. Chem. **73**: 81, 1927.
4. Chaddock, R. E.: Principles and Methods of Statistics, New York, 1925, Houghton Mifflin Co., pp. 241-243.

MAINTAINING THE POTENCY OF BACTERIOPHAGE PREPARATIONS STORED FOR LONG PERIODS OF TIME*

Preliminary Observations

Martha Applebaum, A.B., M.S., and Frances C. Frisbee, A.B.
New York, N. Y.

INTRODUCTION

THE problem of keeping bacteriophage preparations over long periods of time is of interest to laboratory technicians whose responsibility it is to have available at any time a quantity of the lytic principle in proper medium for use, packaged in appropriate containers. It is also of interest to clinicians, especially those remote from the source of supply, in forming a judgment as to the suitability for use of a preparation acquired some time previously.

The following experiments were undertaken in an attempt to determine the loss in activity of three common races of bacteriophage in a variety of containers, stored over a period of one year at icebox and room temperatures, in the two types of medium used routinely in this laboratory.

EXPERIMENTAL DATA

Staphylococcus, streptococcus, and colon bacillus bacteriophages were used in these experiments since they are the types most commonly employed in therapy. The regular stock preparations in broth and asparagin media[1] were

*From the Department of Pathology and Bacteriology, New York Post-Graduate Medical School and Hospital, Columbia University.
Received for publication, November 14, 1938.

tested for potency according to the fluid titration method of d'Herelle.[2] They were then packaged in the six types of container which are used routinely. These include sealed glass ampoules and a variety of glass tubes and bottles protected with cotton and rubber stoppers. One complete set of preparations was stored in the icebox at approximately 9° C. A duplicate set was left at room temperature protected from direct sunlight. No attempt was made to regulate the temperature of this latter set, allowing it to be exposed to the normal variations encountered in the laboratory. It must be borne in mind, in connection with these experiments, that the bacteriophage was prepared and packaged during the month of January, so that the highest temperatures were encountered after six months' storage.

The potency of the bacteriophage thus stored was ascertained at the end of one month. The bacteriophage had been stored in separate tubes for each determination to be done, so that at no time was it necessary to re-enter a tube for testing. The fluid titration method of d'Herelle was used throughout these experiments for determination of potency. A great deal of care was taken to avoid any variation of technique in these many titrations, but, despite this care, small irregularities appeared which could only be credited to technical error. It was, therefore, decided to disregard the difference of one logarithmic quotient in interpreting results.

A determination of potency was made subsequently at the end of three months, six months, nine months, and twelve months.

TABLE I

	STAPHYLOCOCCUS BROTH BACTERIOPHAGE		COLON BACILLUS BROTH BACTERIOPHAGE		STREPTOCOCCUS BROTH BACTERIOPHAGE	
	ICEBOX	ROOM TEMP.	ICEBOX	ROOM TEMP.	ICEBOX	ROOM TEMP.
Original titer	10^{-9}	10^{-9}	10^{-10}	10^{-10}	10^{-9}	10^{-9}
End of 1 month	10^{-9}	10^{-9}	10^{-10}	10^{-10}	10^{-9}	10^{-8}
End of 3 months	10^{-9}	10^{-8}	10^{-10}	10^{-9}	10^{-8}	10^{-5}
End of 6 months	10^{-8}	10^{-4}	10^{-9}	10^{-7}	10^{-8}	10^{-6}
End of 9 months	10^{-8}	10^{-1}	10^{-9}	10^{-6}	10^{-8}	10^{-3}
End of 12 months	10^{-8}	Neg.	10^{-9}	Neg.	10^{-8}	10^{-2}
	STAPHYLOCOCCUS ASPARAGIN BACTERIOPHAGE		COLON BACILLUS ASPARAGIN BACTERIOPHAGE		STREPTOCOCCUS ASPARAGIN BACTERIOPHAGE	
	ICEBOX	ROOM TEMP.	ICEBOX	ROOM TEMP.	ICEBOX	ROOM TEMP.
Original titer	10^{-9}	10^{-9}	10^{-9}	10^{-9}	10^{-9}	10^{-9}
End of 1 month	10^{-9}	10^{-8}	10^{-9}	10^{-9}	10^{-9}	10^{-9}
End of 3 months	10^{-8}	10^{-8}	10^{-9}	10^{-8}	10^{-9}	10^{-8}
End of 6 months	10^{-9}	10^{-6}	10^{-8}	10^{-8}	10^{-9}	10^{-8}
End of 9 months	10^{-9}	10^{-2}	10^{-9}	10^{-7}	10^{-9}	10^{-2}
End of 12 months	10^{-9}	10^{-1}	10^{-9}	10^{-5}	10^{-9}	10^{-2}

A comparison of the results obtained on titrations indicated a negligible difference due to the various containers used for storing the bacteriophage. Table I shows an average of the six separate readings for different types of containers. It shows the original titer of each of the three types of bacteriophage prepared in broth and asparagin media. It then records the average variations for the six types of container at stated intervals over a period of twelve months.

RESULTS

It will be noted from these findings that the bacteriophage stored in the icebox maintained its potency for a period of a year. There was a slight drop in titer evident in the broth preparations after being stored for six months. This is only a drop of one logarithmic quotient, but it seems valid in view of its regularity. The asparagin preparations maintained their original potency throughout the test period.

In the bacteriophage preparations which were stored at room temperature, however, a decided loss in potency will be noted after three to six months. This loss in titer appeared somewhat irregularly, but was evident earlier in the broth preparations than in the asparagin for each type of bacteriophage. The potency was steadily reduced on further standing at room temperature so that in some instances a complete loss of activity was found after twelve months.

DISCUSSION AND COMMENT

These results may fail to settle many small points in regard to the exact length of time during which a certain race of bacteriophage may be left at a given temperature without loss of potency. They do, however, indicate the general trend of loss in lytic activity over fairly long intervals at widely spaced variations of temperature and have a practical application for the laboratory technician and the clinician.

Two points become increasingly obvious as these results are studied. The primary one is that bacteriophage may be stored for as long as one year at icebox temperature without danger of significant loss in activity. To maintain fully potent stock preparations, however, the bacteriophage should be freshly prepared at intervals of not more than six months. The second important finding is that bacteriophage stored at ordinary room temperatures will show a diminishing potency over short periods of time. It is probable that a loss in potency occurs more rapidly at temperatures slightly elevated over the normal room temperature, but this point should be further investigated. It becomes apparent that bacteriophage may be left at room temperature for adequate sterility determinations. Beyond the time necessary for these tests it is not wise to leave the bacteriophage preparations exposed to other than controlled icebox temperatures.

CONCLUSIONS

It is concluded that bacteriophage preparations may be left for periods of at least a year at icebox temperature without seriously affecting the lytic activity. It is also concluded that after a period of three months at a temperature of 20° C. or above, a loss of activity of the bacteriophage becomes apparent, followed in some instances by a complete loss of such lytic activity.

Acknowledgment is made to the able assistance of Marie Wasseen and Margaret McRae in carrying out this work.

REFERENCES

1. Morton, Gladys, and Wasseen, Marie: The Technique for Isolation and Preparation of Bacteriophage, J. LAB. & CLIN. MED. 20: 1188, 1935.
2. d'Herelle, F.: The Bacteriophage and Its Behavior, Baltimore, 1926, Williams & Wilkins, p. 77.

THE DETERMINATION OF VITAMIN C IN URINE*

HARRY N. HOLMES, Ph.D., AND KATHRYN CAMPBELL, OBERLIN, OHIO

THERE is an increasing interest in the daily urinary excretion of vitamin C (cevitamic or ascorbic acid) since many infections, if not most, are accompanied by considerable diminution in such excretion. It would seem that under such circumstances the patient should have the optimum vitamin C level in his body maintained by proper diet or dietary supplement. Otherwise something approaching subclinical scurvy may conceivably become an annoying complication.

It is unfortunate that the oxidation losses in twenty-four-hour specimens as collected in actual practice are often so great as to mislead the physician in his diagnosis.

Using the Harris and Ray[1] method of titration against a fresh solution of the dye, 2,6 dichlorophenolindophenol, we determined the cevitamic acid content of fresh urine and of the same specimen after twenty-four hours. For this particular experiment the urine was kept in a clear glass bottle, open to the air. The loss (by action of light and oxygen) was 100 per cent. King and others attempted to preserve the vitamin by the aid of acetic acid (8 per cent of the total volume) and metaphosphoric acid (2 per cent of the total), or metaphosphoric alone, and gained some degree of success by so doing. We tested the inhibiting effect of acetic acid (10 per cent of total volume, as effective as the two acids together) and found a loss of 46 per cent of the vitamin in twenty-four hours. Even in twelve hours a 32 per cent loss was observed. To be sure, this was in warm summer weather with greater speed of oxidation than would be observed at lower temperatures. The open bottle test was severe. Yet air in partly filled bottles must be considered.

It is not permissible to get around this loss by analyzing a single specimen of freshly voided urine because the vitamin C content of urine voided at different times in the day or night varies greatly. If a considerable amount of the pure vitamin is given to a healthy person, saturated with the vitamin, urine excreted within an hour or two may contain the greater part of the extra dose. Evidently the safer course is to collect and preserve properly the four or five specimens usually voided during twenty-four hours. The patient must be warned that this does not mean beginning at 7:00 A.M. and finishing with another 7:00 A.M. specimen. He should discard either the first or the last 7:00 A.M. urine.

If the usual twenty-four-hour collections were in the physician's hands six hours after the end of the period and he was free to make an analysis at once

*From Oberlin College, Oberlin.
Received for publication, November 14, 1938.

(often impossible), the average loss in warm weather might approximate 40 per cent of the vitamin originally present in the total excretion.

We decided to improve the methods of preserving urine during the collection period and the hours of delay in analysis.

Immediate chilling of each specimen was a distinct help, but impracticable. In whose refrigerator would bottles of urine be welcomed?

Exclusion of light by use of brown bottles proved to be a help in protecting the vitamin, but this was not enough.

A bottle filled to the cork was preferable to one containing a considerable air space, but collections are not usually of any desired volume. Partly filled bottles will be the rule, not the exception. Furthermore, in the act of voiding urine into a bottle some air is dissolved.

Common corks are porous enough to admit air on several hours' standing, so we finally selected brown bottles closed with screw caps of plastic, not metal. Rusty metal caps catalyze oxidation of vitamin C.

Exclusion of air by carbon dioxide was successfully achieved by dropping small fragments of dry ice, solid carbon dioxide, into each bottle as urine was voided. The effect was excellent due to chilling and exclusion of air, but sometimes too much pressure was set up after closing the bottle. The inconvenience of securing the dry ice is a strong factor against its use.

Finally we adopted the plan of adding a slightly rounded teaspoonful (about 3 gm.) of precipitated calcium carbonate (a fine dry powder) to the bottle of fresh urine into which 50 c.c. of glacial acetic acid had already been measured. After about half a minute of frothing to permit escape of air and carbon dioxide, the cap was screwed very tight. The escaping carbon dioxide drove out dissolved air as well as free air in the bottle. The resulting success suggested a final precaution: the extra addition of a fragment of marble, the size of one or two grains of corn (about 2 gm.). The quick action of the powdered calcium carbonate on the acetic acid was effective before capping the bottle while the slow action of the fragment of marble continued for several hours, setting up a small positive pressure of the gas, very helpful if the cap or stopper was not airtight.

The astonishing gain in accuracy due to this simple method of collection is shown in Table I.

TABLE I

LOSS OF VITAMIN C IN URINE UNDER VARIOUS CONDITIONS OF COLLECTION
(Fresh Urine in 500 c.c. bottle, only partly filled, warm weather)

BOTTLE, 500 C.C.	ACID	$CaCO_3$	COVER	TIME	LOSS OF VITAMIN IN PER CENT
Clear glass	None	None	Open	24 hr.	100
Clear glass	10% acetic acid	None	Open	24 hr.	46
Clear glass	10% acetic acid	Fragments	Screw cap too quickly closed	24 hr.	20
Brown	10% acetic acid	Fragments	Screw cap too quickly closed	24 hr.	3
Clear	10% acetic acid	Powder and fragments	Capped after half minute	24 hr.	None
Brown	10% acetic acid	Powder and fragments	Capped after half minute	48 hr.	None

Using the chemist's Bunsen valve, which permits gases to pass out from the bottle but allows no air to enter, we found no measurable loss in forty-eight hours. This device, with small lumps of dry ice, is perfect but rarely usable for the average patient.

CONCLUSIONS

Suitable 500 c.c. amber bottles with screw caps of plastic (no metal) may be bought through dealers who in turn may buy from the Libby Owens Illinois Glass Co., of Toledo. Two types of bottles, the narrow and wide mouth, are needed.

Mold A-6338, 500 c.c. (16 oz.) narrow mouth, round, amber glass, 28 mm. 0.1 G-419 finish. Black molded caps—18 mm.—419 with cork and vinylite liners. Mold A-5160, 500 c.c., wide mouth, amber glass, 53-400 finish. Black molded caps—53 mm.—400. Pulp and vinylite liners.

To simplify collections the 3 gm. of dry precipitated calcium carbonate powder and a 2 gm. fragment of marble should be placed in a sealed envelope attached to the bottle by band or cork. The patient should be instructed to allow frothing to continue fully half a minute (to insure displacement of air by carbon dioxide) before tightly closing the bottle. Bed patients should void into some other vessel due to the possibility of acid burns.

If the foregoing directions are followed, urine may be preserved forty-eight hours without appreciable loss of vitamin C. Otherwise half or all of the vitamin may be lost in twenty-four to thirty-two hours.

TITRATION PROCEDURE

Mix rapidly contents of all the bottles representing twenty-four hours' collections, measure with a 500 c.c. graduate, and pour a small sample quickly into a 2 c.c. burette, graduated in hundredths, keeping the stopcock open until the liquid just reaches the stopcock. Wait nearly one minute or until the carbon dioxide bubbles rise and escape before again opening the stopcock and filling the burette to the tip. Run out liquid until the upper level is at the zero or starting point on the scale. Discard any urine escaping from the tip at this stage.

Titrate the urine into a 15 c.c. conical centrifuge tube containing 0.05 c.c. of a standard solution of the reducible dye, 2,6 dichlorophenolindophenol until the pink color following the blue of the dye weakens and becomes barely noticeable, remaining so for thirty seconds. It is helpful in this color comparison to hold a similar centrifuge tube of urine, diluted to the same extent, beside the sample in titration. The end point is secured when only a very slight difference in tint appears (against a white paper background).

Hoffmann-La Roche, of Nutley, N. J., sell conveniently standardized dye tablets, each one sufficient to react with 1 mg. of vitamin C.

To make a standard solution, place one tablet in any very small bottle (or 20 c.c. flask) and add one drop less than 2 c.c. of distilled water from a 2 c.c. pipette. This allows for the slight increase in volume of the solution. Allow to stand a few hours to secure complete solution of the dye.

A daylight lamp is an aid in accurate color readings. Not more than two minutes' time is allowable for a single titration.

Calculation.—For example, suppose that it required 1.4 c.c. of urine of a 500 c.c. specimen to decolorize 0.05 c.c. of the standard, then

1.4 c.c. urine should contain 0.025 mg. of vitamin C.

The 0.05 c.c. of dye solution is $\frac{1}{40}$ of the 2 c.c. which was found to be equivalent to 1 mg. of vitamin C.

$$\text{Therefore, 1 c.c. urine contains } \frac{0.025}{1.4} \text{ mg.} = 0.01785 \text{ mg.}$$

$$\text{and 500 c.c. contains } 500 \times 0.01785 \text{ mg.} = 8.9+ \text{ mg. vitamin C.}$$

Other reducing substances often present during illness may introduce some error, but efforts to remove them introduce other errors.

Titration Materials.—The materials necessary for titration include: Microburette, 6-414-J, 2 c.c. graduated in 0.01 c.c. divisions, funnel top. Fischer Scientific Co., Pittsburgh. Pipette, 13-674, capacity 0.1 c.c., graduated in 0.01 c.c. divisions, Fischer Scientific Co. Pipette, 2 c.c. Mixing bottle, 2 liters. Burette stand, small size. Graduate, 500 c.c., to measure total urine volume. Beaker, 50-100 c.c. Dye tablets of 2,6 dichlorophenolindophenol, Hoffmann-La Roche, Nutley, N. J. Glacial acetic acid (50 c.c. for each specimen). Marble, fragments of 2 gm. each, size of grain of corn. Calcium carbonate powder (precipitated).

REFERENCE

1. Hawk: Practical Physiological Chemistry, ed. 11, Philadelphia, 1937, P. Blakiston's Son & Co.; Lancet **228**: 71, 1935.

A SIMPLE METHOD FOR RECORDING SPLENIC VOLUME CHANGES IN THE INTACT, UNANESTHETIZED DOG*

Effects of Epinephrine, Pilocarpine, Atropine, and Benzedrine on Splenic Volume

Laurence L. Palitz, A.B., Ph.D., Chicago, Ill.

UNTIL 1925 work on the volume changes of the spleen was limited to oncometric studies done in acute experiments on animals under anesthesia. In that year Hargis[1] and Barcroft[2] devised methods for determining volume changes in the intact, unanesthetized dog. The former placed aseptically around the spleen a collodion-gauze plethysmograph which had an outlet to the exterior attached to a water manometer. Barcroft at first inserted a window in the wall of the abdomen of dogs for direct observations on splenic volume, but he later discarded this procedure for two others which were more effective: (a) In aseptic preparations lead pellets were sewn around the margin of the spleen. After recovery of the animal observations on volume changes were made by taking x-ray photographs. (b) After complete exteriorization of the spleen and subsequent recovery the spleen was outlined from time to time on a celluloid plate.

*From the Department of Physiology, University of Chicago.
Received for publication, December 1, 1938.

The methods most commonly used are the two last described because of the almost insuperable difficulty of the Hargis technique. Although these procedures are accurate and relatively simple, they are subject to criticism on the score of practicability. Expense limits the x-ray method to very few laboratories. Both methods fail to give uninterrupted, continuous records of spleen volume, and are thus unsatisfactory for studying the effects of drugs or for any other problem where it is desirable to know at what point the spleen is most contracted and how long the contraction lasts. It was, therefore, deemed advisable to devise a method at once simple and inexpensive, whereby continuous, uninterrupted records could be obtained.

METHOD

Under ether anesthesia, and with aseptic precautions, a laparotomy is made in the left hypochondrium above the spleen. After ligating and severing the upper and left part of the gastrosplenic omentum to give freedom of movement, the spleen is brought out to the surface. The omentum is drawn out and held in place around the spleen by a few stitches. A space between the external oblique and the skin is made with a finger and the spleen placed into it, care being taken to allow sufficient room for the vessels to lie unobstructed. The skin is then sewed over the spleen. Thus the animal is prepared with a subcutaneous spleen. The vessels and nerves are untouched, and the spleen functions quite normally outside the abdominal cavity, yet protected from the exterior by a thin layer of skin.

After allowing sufficient time for recovery, the dog is trained to lie quietly on a table. A sphygmomanometer cuff is then wrapped tightly around the abdomen with the bladder of the cuff enclosing the spleen. The cuff is held in place with a few hemostats. For obvious reasons care should be taken to make the cuff as immovable as possible. One outlet of the bladder is attached to a water manometer; through the other, a measured amount of air is introduced and the outlet is clamped off. Splenic volume changes are determined by noting changes in the level of the record. A simultaneous respiratory record is taken to rule out changes which might be due to respiration.

RESULTS

The records reveal slight rhythmical contractions of the spleen occurring every minute. After injection of epinephrine these rhythmical contractions are very much accentuated (Fig. 1), confirming the findings of Barcroft.[4]

Epinephrine hydrochloride, a drug known to contract the spleen, was injected in order to test the sensitivity of the apparatus. Two cubic centimeters of a 1:100,000 solution, administered intravenously, caused a consistent "drop" in the normal splenic volume of 27.3 mm. (average of 13 experiments; Fig. 1). The splenic volume returned to normal after 16.2 minutes (average).

As a control 2 c.c. of saline were injected (Fig. 2). No significant change in the level of the record occurred. The slight rise recorded is a mechanical effect due to movement of the animal's leg during the injection. To rule out possible abdominal changes, the bladder of the cuff was placed on the side of

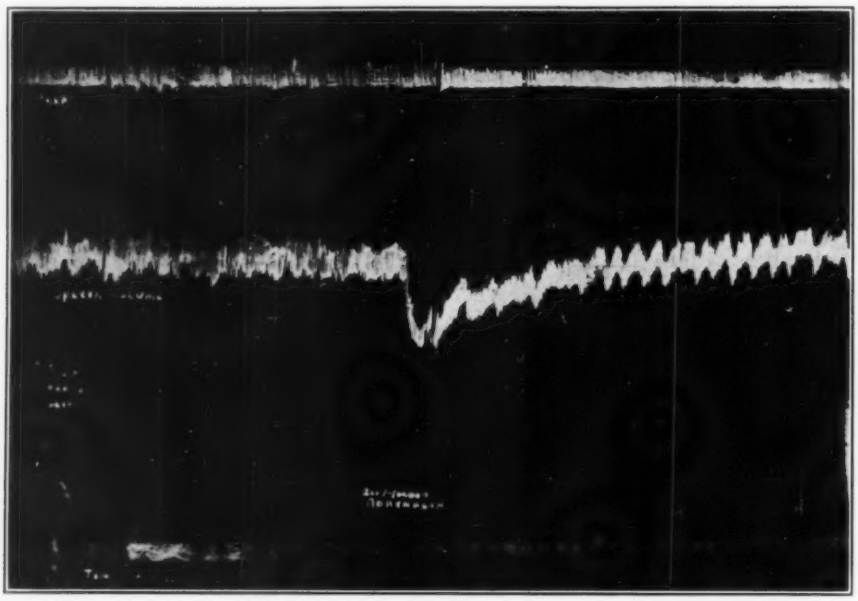


Fig. 1.—Record of the effect of 2 c.c. epinephrine (1:1,000,000) on splenic volume. Note the accentuated one-a-minute contraction after epinephrine injection.

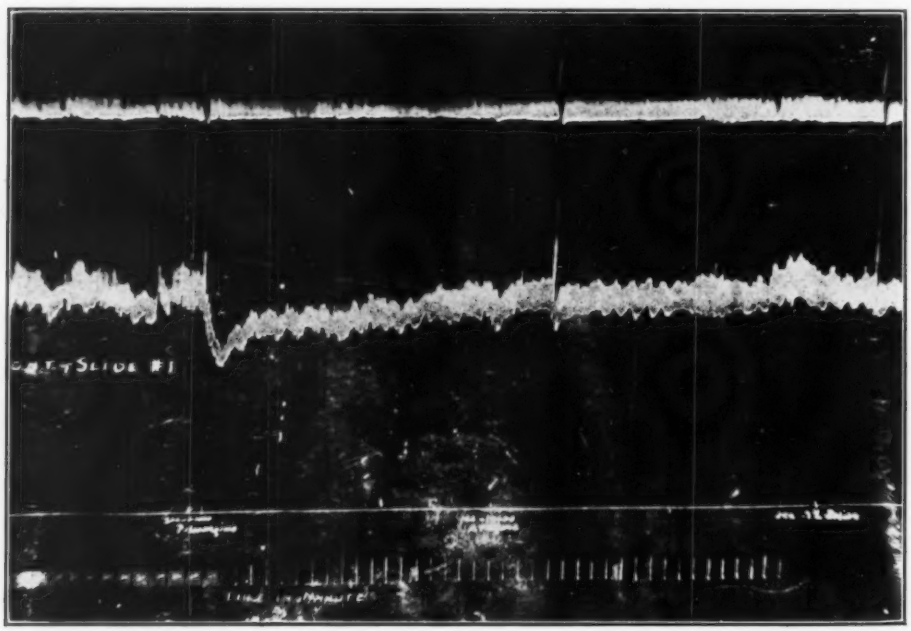


Fig. 2.—The effects of 2 c.c. pilocarpine (1:1,000), 1 c.c. atropine (1:1,000), and saline on spleen volume. The slight rise with saline is a mechanical effect due to movement of the leg with injection.

the abdomen away from the spleen, and 2 c.c. of 1:100,000 epinephrine were injected. Again there was no drop in the level of the record (Fig. 3).

Pilocarpine (2 c.c. of 1:1000) caused a contraction almost as marked as that caused by epinephrine (20.1 mm. drop; average of 6 experiments). However, the duration of contraction was lengthened; invariably it was at least

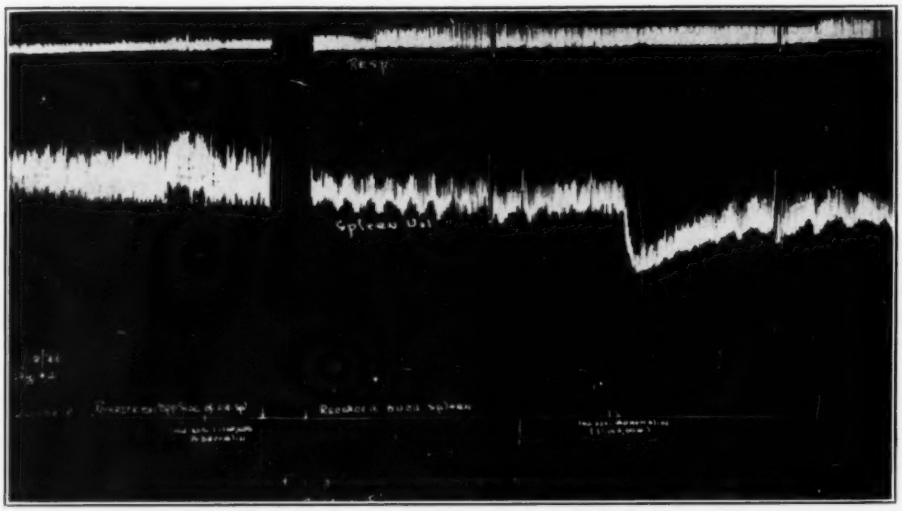


Fig. 3.—Record of epinephrine injection with the rubber bladder on the opposite side of the abdomen, followed by the same quantity of epinephrine with the bladder over the spleen.

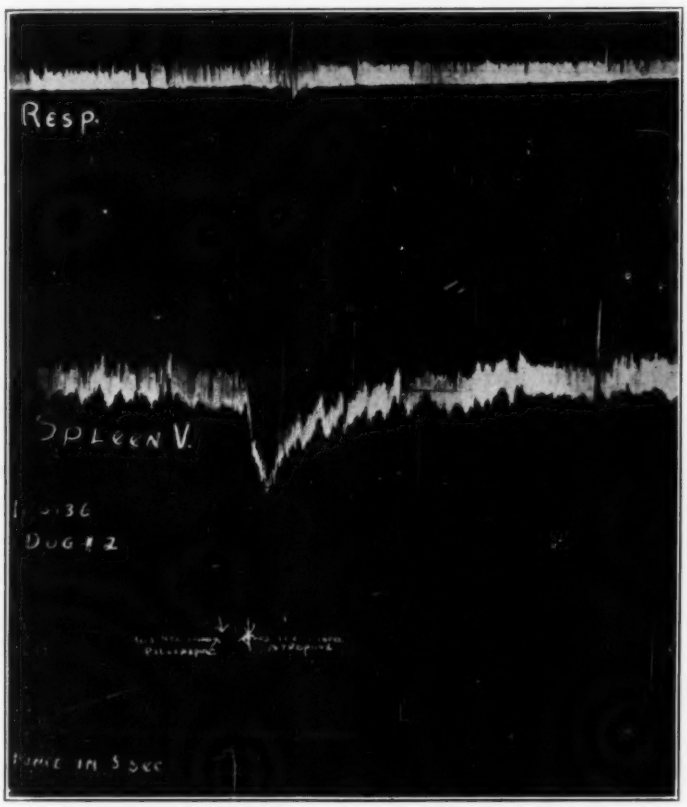


Fig. 4.—Effects of injection of pilocarpine immediately followed by injection of atropine. The return to normal is tremendously accelerated.

thirty minutes. The antagonism between pilocarpine and atropine is brought out by the fact that when atropine was administered immediately after pilocarpine, the spleen returned to normal in seven minutes (Fig. 4); and further by the fact that atropine (2 c.c. 1:1000), injected before pilocarpine (2 c.c. 1:1000), completely abolished the pilocarpine effect. Atropine alone was without effect.

Ten milligrams benzedrine were injected intravenously, causing a significant contraction (20 mm.), slow in onset and longer in duration. After five hours the spleen had not yet come back to normal (Fig. 6).

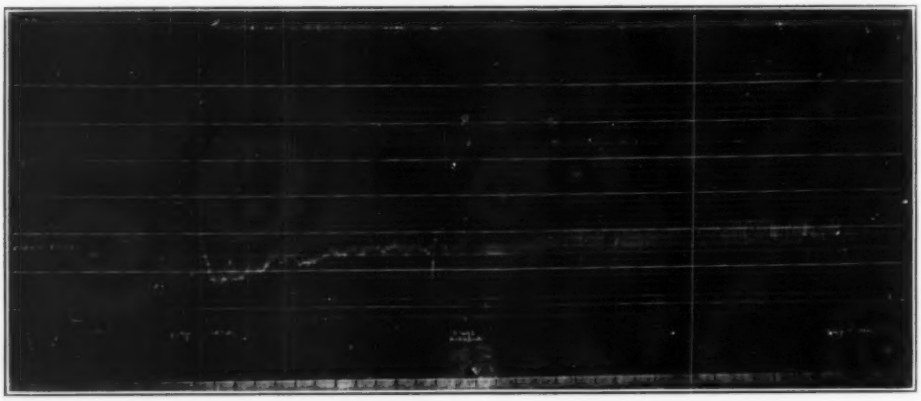


Fig. 5.—Effects of intravenous injections of 3 mg. of pilocarpine before and after injection of 3 mg. of atropine.

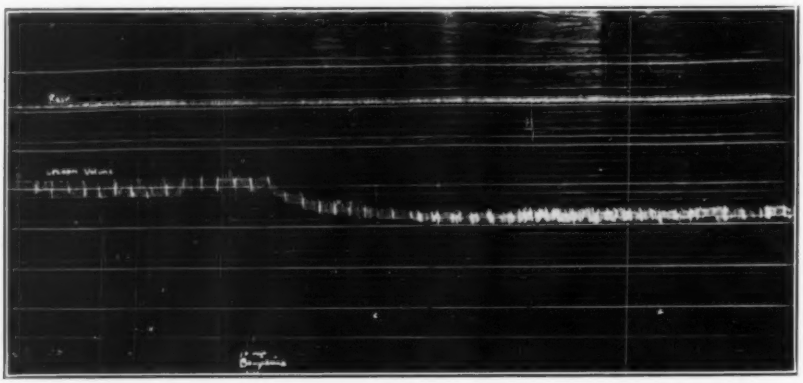


Fig. 6.—Effect of intravenous injection of 10 mg. of benzedrine. After five hours the spleen had still not returned to normal.

DISCUSSION

Although these results are mainly confirmatory, they point out the adequacy of the method. However, there are some limitations. For drug work, where the animal can be trained to lie quietly while the drugs are being injected, this method is satisfactory and preferable to the others in that a continuous, uninterrupted record can be obtained; it is extremely simple in execution and once the preparation of the animal is complete, extra care is unnecessary. On the other hand, for experiments in which the animal is in motion, such as in conditions of exercise, the difficulty in keeping the sphygmomanometer cuff immobile makes preferable the more direct methods of Barcroft.

The benzedrine action has not been previously reported to our knowledge, but its effect was not surprising because of its close chemical relationship to ephedrine and the epinephrine-like compounds.

SUMMARY

(1) A simple method is described whereby continuous, uninterrupted records of spleen volume changes can be repeatedly obtained in the intact, unanesthetized dog. The subcutaneous spleen has more nearly normal surroundings than does the exteriorized spleen; once it is placed under the skin, extra care of the animal is unnecessary.

(2) Confirming the work of many authors on anesthetized and unanesthetized animals, epinephrine and pilocarpine both significantly contracted the spleen. Atropine injected alone was without effect; but when injected after pilocarpine, it accelerated the return to normal. Atropine, injected before pilocarpine, abolished completely the pilocarpine effect.

(3) Benzedrine caused a prolonged contraction of the spleen with relatively slow onset.

It has been a great privilege and opportunity to carry out this work under the direction of Dr. Arno B. Luckhardt.

REFERENCES

1. Hargis, E. H.: A Method for the Study of the Variations in Volume of Organs in the Intact Animal, J. Lab. & Clin. Med. **11**: 664, 1926.
2. Barcroft, J.: Recent Knowledge of the Spleen, Lancet **1**: 319, 1925.
3. Barcroft, J., and Elliot, R. H. E.: Some Observations on the Denervated Spleen, J. Physiol. **87**: 189, 1936.
4. Barcroft, J., and Nisimaru, Y.: Cause of the Rhythmical Contraction of the Spleen, J. Physiol. **74**: 299, 1932.

THE TIME INTERVAL IN READING THE KAHN TEST*

Elizabeth C. Brown, A.B., and Nathan Nagle, A.B., St. Louis, Mo.

THE serologic surveys conducted by the United States Public Health Service established that the sensitivity of the Kahn and other serologic tests, as conducted in different laboratories, showed considerable variation. Several known factors which influence the Kahn test, viz., sensitivity of antigen, time and temperature of heating sera, and shaking time and speed, have been standardized. Other factors, such as the time interval in reading the test after the addition of physiologic salt solution following the shaking period, have not been satisfactorily standardized.

Recently, it has been observed in this laboratory that the time interval in reading the Kahn test definitely influences the sensitivity of the test. This observation was made by reading the results immediately after adding the physiologic salt solution to a group of 142 tests and repeating the readings

*From the Laboratory Section, St. Louis Health Division.
Received for publication, December 1, 1938.

ten minutes after the addition of salt solution. The sera employed in these tests were secured from syphilitic patients receiving treatment. It was found that the second reading showed a reduction in sensitivity of about 5 per cent as compared with the first reading.

This finding suggested the undertaking of a more elaborate experiment along the same line. Accordingly, 1,500 routine Kahn tests were read at the following three intervals: immediately after the salt solution was added (following shaking), and ten minutes and twenty minutes after the addition of salt solution. The salt solution was added to 30 tests at a time.

Of the 1,500 tests made 481 sera gave positive or doubtful results when read immediately or during the two other intervals, while 1,019 tests were negative.

TABLE I

KAHN TESTS (481) READ IMMEDIATELY AFTER ADDING SALT SOLUTION, TEN AND TWENTY MINUTES LATER

NUMBER OF TESTS	IMMEDIATE READINGS	NUMBER OF TESTS WITH CHANGES IN READINGS AFTER 10 MINUTES						TESTS WITH FURTHER CHANGES IN READINGS AFTER 20 MINUTES					
		++++	+++	++	+	±	−	++++	+++	++	+	±	−
298	++++		10*	3					4	3*			
79	+++	5		18	4	1				6	1		
56	++		5		15	2	2				2		1
37	+			2		1	15			1		1	
7	±				1		2						1
4	−					1†					1†		
		Positive		Doubtful		Negative		Positive		Doubtful		Negative	
433	Positive (++++, +++, ++)			22		2				3		1	
44	Doubtful (+, ±)	2				17		1				1	
4	Negative			1						1			

*Two ++++ readings became +++ at ten minutes and ++ at twenty minutes.

†One originally negative reading became ± on standing ten minutes and + on standing twenty minutes.

The results summarized in Table I indicate the changes that occurred in the 481 specimens giving positive or doubtful readings, based on the average of the reactions in the three tubes. A comparison can readily be made between the final reports based on the use of the symbols: ±, +, ++, +++, ++++, and the recommended method of negative, positive, or doubtful reports wherein ++ and above are positive and + and ± are doubtful. In Table I the numbers to the right of the diagonal line represent number of tests in which readings have become weaker and those to the left of the line indicate tests in which readings have become stronger.

When changes occurred after the first reading, the tendency was for the results to become weaker. However, in 16 specimens the results became stronger. It should be noted also that increases in readings made in ten minutes were

not lost at twenty minutes. The results indicate that 18.1 per cent of the readings changed during the ten minutes after the salt solution was added to the test, while 22.7 per cent had changed readings during the twenty-minute interval.

Of the 18.1 per cent of readings that changed within ten minutes, only 3.4 per cent became stronger, while only 3.8 per cent of the 22.7 per cent of tests showing changes during the twenty-minute period became stronger. It is important to note that 22, or 5.1 per cent, of 433 final "positive" reports changed to final "doubtful," and 2 changed to final "negative" reports when read ten minutes after the addition of the salt solution. Furthermore, 18, or 4.1 per cent, of the tests changed from final "doubtful" to "negative" reports when read after twenty minutes.

DISCUSSION

The present study suggests that a uniform time interval should be employed for reading the Kahn test, particularly when several laboratories are testing specimens of the same sera for standardization purposes. In this connection it may be well to quote the following personal communication to the authors from Dr. Kahn: "For a number of years we have recommended that the Kahn tests be read immediately after the addition of salt solution (following the three minutes' agitation) and reread 15 minutes after the addition of salt solution. If there is any variation in the results of these two readings, the average of the results should comprise the final report. Variations occur generally in sera from patients undergoing treatment especially when these sera give borderline reactions." From our observations in treated cases of syphilis, the higher reading obtained seems to be the most significant. We have attempted to modify the salt solution so that floccules obtained in positive sera would remain stable. So far we have used a series of buffered water with pH ranging from 4.0 to 9.0, and various concentrations of glycerol have been added to salt solution without any success.

CONCLUSIONS

Before comparable results can be obtained with the Kahn test in two or more laboratories, even though the standard technique is followed and the same lot of antigen used, it is necessary to standardize the time factor in reading the test. If the test is read immediately after the addition of salt solution, a larger number of "positive" reactions will be found than if the test is read ten or twenty minutes after the addition of salt solution.

A NOTE ON A SIMPLE METHOD FOR THE CLARIFICATION OF MEAT INFUSION*

AARON H. STOCK, M.D., PITTSBURGH, PA.

MEAT infusions are usually clarified by filtration through a paper filter or meat on a colander, or by the use of egg white. These methods are sometimes undesirable, and are usually cumbersome, especially in the preparation of 30 to 40 liter lots of meat infusion.

Large quantities of clear meat infusion may be made rapidly without transfer of the meat or the use of paper or egg white if one has access to a large kettle equipped with a pipe outlet at the bottom. We have used a 60 gallon steam-jacketed, copper kettle lined with block tin. The kettle has an outlet at the bottom which takes a vertical pipe, 1 inch in diameter and 3 inches in length. Joined at right angles to the vertical pipe is a horizontal section, 12 inches long, equipped with a brass faucet.

For every 450 gm. of meat in the kettle, 1 liter of water is added. The mixture is boiled for thirty minutes and then allowed to settle. The faucet is opened and the infusion allowed to run out slowly. The pipe outlet becomes filled with meat and soon acts as a perfect filter.

As an added precaution against the possible passage of particles, the fluid may be allowed to flow through a small mat of pyrex glass wool on a colander placed above the receiving vessel.

NOTE ON A NEW METHOD FOR THE COLLECTION OF URINE IN DOGS†

B. LIBET, B.S., AND L. YESINICK, B.S., CHICAGO, ILL.

THE method now extensively in use for collecting urine in acute mammalian experiments involves cannulating the ureters with long ureter cannulae (by the trochar technique).[1] This method, although satisfactory, is time-consuming and somewhat difficult. Pains must be taken to insert the cannulae in the proper direction, for if they are not introduced parallel to the course of the ureters, kinking of the latter occurs. The method here presented was used in a large number of acute experiments in dogs in which it was desired

*From the Pathological Laboratories, University of Pittsburgh, and the Children's Hospital, Pittsburgh.
Received for publication, December 8, 1938.
†From the Physiological Laboratories of the University of Chicago.
Received for publication, December 10, 1938.

to collect urine, and was found to be simpler and less time-consuming. Essentially, it consists of introducing a bladder tube into the animal's bladder.

The bladder tube consists of a hollow spherical glass bulb, 2 to 5 cm. in diameter, and a glass tube of 2 mm. bore, about 25 cm. in length, the bore of which is continuous with the inside of the bulb. The bulb is perforated with twelve or more holes of 4 mm. bore. The tube is bent 30° from the horizontal at about 10 cm. from the bulb (see Fig. 1).

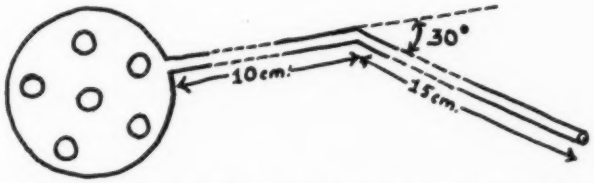


Fig. 1.—Diagram of bladder tube (modified Folin tube).

To insert the bladder tube into the bladder, make a 5 cm. incision along the linea alba just anterior to the genitalia in the female, and lateral to the penis in the male. Expose the bladder and carefully isolate the ureters for about 2 cm. from their entrance into the bladder. Introduce a strong ligature under the isolated ureters, bringing the ends of the ligature around the urethra, and tie tightly. The ligature should include all the tissues leading to the bladder except, of course, the ureters. It is important that this step be performed carefully, for it serves a double purpose: (1) to tie the urethra so that no urine can escape from the bladder except via the bladder tube, and (2) to choke off the blood supply of the bladder. If the blood supply is not adequately cut off, bleeding may occur from the cut edge into the bladder, and the blood will appear in the urine. Make an incision in the ventral wall of the bladder, being careful to cut only the bladder musculature and leaving the mucosa intact. (The layers separate easily.) Control hemorrhage, if any. The mucosa is then cut through and the bulb of the bladder tube introduced into the bladder. The incision in the bladder is closed tightly around the emerging tube by two hemostats or by sewing together the cut edges.

If the tube does not fill spontaneously, it is filled with fluid as follows: the needle of a syringe containing warmed Ringer's solution is introduced through the bladder wall, through a hole in the bladder tube, into the bulb. The air is driven out as the liquid is forced through the tube.

The animal may be turned on its side to facilitate the urine collection. Drops may be recorded in the usual manner.

Since the bladders of different dogs vary in size, different sized bulbs must be used. We have not found it necessary to use more than three different sized bulbs: bulbs of 2 cm., 3.5 cm., and 5 cm. diameters. In any case, the bulb need not be nearly as large as the bladder, since the bladder contracts considerably when the bulb is introduced. The bulb should be of such a size that, after the bladder has contracted, there should be a snug fit. If flow does not take place it may be due only rarely to blocking of the ureters by the glass bulb; this is remedied by turning the glass bulb slightly.

Because of its relative simplicity, this method is especially recommended for class work on the mammalian urine experiments, and research experiments concerned with rate of urine flow.

We wish to express our thanks to Dr. A. B. Luckhardt, who suggested the principle of this method to us.

REFERENCE

1. Luckhardt, A. B.: Note on a Uniformly Satisfactory Method of Collecting Urine Separately From Each Ureter in Acute Experimental Work (Dogs), J. Lab. & Clin. Med. 3: 558, 1918.

A SIMPLE AND INEXPENSIVE CAGE FOR MICE*

Florence L. Evans, Ph.D., Carville, La.

THE cage herein described can be adapted either to animal inoculation experiments or, in a slightly more elaborate form, to dietary experiments as metabolism cages.

The cage consists of a number 10 or gallon tin can fitted with a cover of hardware cloth and galvanized iron. The can is placed on its side in a wooden rack which keeps it from rolling. Ventilation is provided by holes punched in the sides and top. One larger hole in the top admits the water tube. The cap of galvanized iron and hardware cloth may be made to fit snug enough so that a mouse cannot push it off, and yet it can be removed readily by hand. If the caps are too loose, they can be fastened by a bent nail or heavy wire dropped through matching holes in the cap and near the edge of the tin can.

If the cage is to be used for metabolism experiments, it is provided with a platform of hardware cloth reinforced at the sides and, if necessary, with one diagonal piece of heavy wire or strip of galvanized iron. It is made wide enough so that it fits in the curve of the can about one-third of the way from the bottom, and long enough so that it comes to the edge of the can; otherwise, the mice will crawl into the bottom. The platform is held, so that it does not slide, by drops of solder on the sides of the can just above the platform.

The racks for the cages consist of two pieces of wood with segments of a circle cut out for the cans to fit in. Matching pieces are fastened together with strips of wood 4½ or 5 inches long. By making additional racks with arcs cut out on the bottom to fit over the first row of cans, and arcs cut out of the top to hold new ones, several layers of cans may be placed together in one unit.

The cans are easily cleaned and can be subjected to sterilization by heat if desired.

When the tin can rusts out, the cap, platform, and racks can be retained, and the can replaced with a fresh one. In use, these cans last about a year before they need replacement.

---

*From the Laboratory of the U. S. Marine Hospital, Carville.
Received for publication, December 12, 1938.

Fig. 1.—The cages assembled.

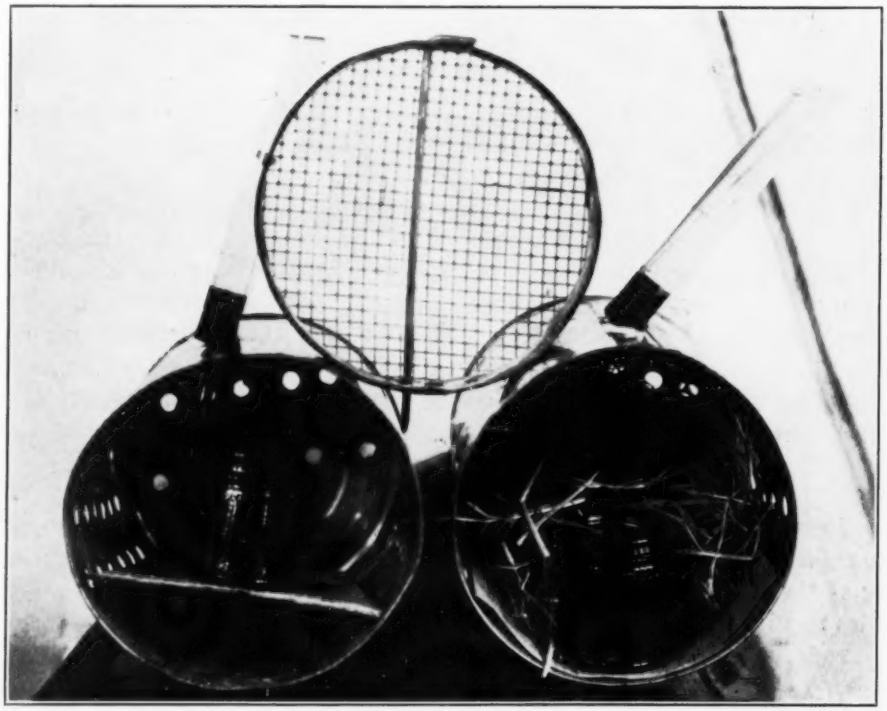


Fig. 2.—The construction of individual cages.

A QUICK AND RELIABLE METHOD FOR STAINING GONOCOCCUS SMEARS*

SETH T. WALTON, Ph.D., CHARLOTTE, N. C.

FOR some time a need has been felt for a staining technique that would speed up the examination of smears for gonococci in the public health laboratory where a great volume of work is always on hand. It is needless to say that accuracy could not be sacrificed because of the already low efficiency of this type of examination.

STAINING SOLUTION

Methyl green, dye content 60 per cent	1.0 gm.
Pyronine, bluish, certified	0.2 gm.
Methyl alcohol, absolute	10.0 c.c.
Phenol, 2 per cent aqueous solution	100.0 c.c.
Glycerol, C.P.	20.0 c.c.

Dissolve dyes in the alcohol and phenol by intermittent shaking for two days, shaking for about two hours each day on a mechanical shaker. Filter and add the glycerin. Refilter into dropping bottle as used.

MODIFIED PAPPENHEIM STAIN

The method here outlined is a modification of the Pappenheim-Saathof formula given in Dr. Conn's book, *Biological Stains*, 1929. The main variation from this formula is the substitution of 10.0 c.c. of absolute methyl alcohol for the 5.0 c.c. of 95 per cent ethyl alcohol. This appears to make a great difference in the stability of the staining solution, since the methyl green used in the formula is a higher homologue of the dye crystal violet with the seventh CH_3 group very loosely attached. When methyl alcohol is a constituent of the solvent, the presence of extra CH_3 groups prevents this seventh methyl group from dissociating itself and leaving a crystal violet molecule. When this occurs, the crystal violet in the mixture intensifies the staining ability of the solution so much that it loses its differential characteristics.

TECHNIQUE

Pass the slide lengthwise through the flame slowly four or five times. Add the stain immediately. The slide should be warm enough to cause slight steaming of the stain. Wash off the stain in from twenty to fifty seconds. Greasy slides require the longer time.

RESULTS

Gonococci take the pyronine readily and stain a deep red color. All other bacteria are stained a pale purplish and are barely noticeable. The nuclei of

*From the City Health Department, Charlotte.
Received for publication, December 13, 1938.

pus cells are green, and the cytoplasm is a soft pink or rose color. With this contrast of colors the gonococci are much more noticeable as the slide is moved under the microscope than on a Gram-stained preparation where pus and epithelium are the same color as the gonococci.

Three years of experience with this technique has convinced me that more positive results can be obtained by its use. The reason is that upon examination of smears, especially vaginal smears, one often finds a single organism, or a single pair, or several single gonococci which are easy to see with the deep-contrasting stain, but which are extracellular and not typically grouped. They do not have enough resemblance to the gonococcus to warrant a positive report, but the finding is sufficient to warrant further search; eventually, a typical nest of gonococci can be found.

It might be stated that this stain is not selective for the gonococcus but for the Neisseria group of microorganisms.

A SIMPLE AND TIME-SAVING PROCEDURE FOR THE IDENTIFICATION OF TREPONEMA PALLIDUM*

M. J. KNISELY, A.B., M.T., TULSA, OKLA.

THERE has been a tendency in the last few years to supplant the carbon arc dark-field method of identification of *Treponema pallidum* from primary lesions. The necessary equipment is expensive, and the preparation laborious and time-consuming.

The following simple method has proved very satisfactory, and has checked consistently with the dark field.

The reagent is a colloidal aqueous solution of silver sold under the nomenclature of Collargolum. It is diluted 1 part in 20 for use, and may be thus purchased.

Place a drop of the suspected material on a clean glass slide. Add an equal amount of the reagent. Mix, and streak out by the use of another slide, exactly as slides are prepared for blood differentials. The preparation dries in a few seconds, with a metallic sheen, and is ready for microscopic examination.

Do not attempt to fix the material on the slide and stain subsequently. The resultant preparation will be too thick for examination.

Microscopically the unstained *Treponema pallidum* stands out distinctly against a background which ranges from dark yellow to light brown.

The entire method and identification in most cases can be done in ten minutes. In addition to the simplicity, slides so prepared may be labeled and kept as permanent records.

---

*From the Morningside Hospital, Tulsa.
Received for publication, December 14, 1938.

THE DIFFERENTIATION OF BOVINE AND HUMAN STRAINS OF TUBERCULOSIS BY THE METHOD OF BERTRAND, BABLET, AND BLOCH*

MARGARET BEATTIE, A.B., AND ROBERT NICEWONGER, A.B., BERKELEY, CALIF.

IN 1937 Bertrand, Bablet, and Bloch[1] described a method for differentiating the human from the bovine type of *Mycobacterium tuberculosis* by intracerebral inoculation of cultures. Their work was based upon observations of Boquet and Broca[2] that bovine strains, when inoculated intracerebrally, produce a fatal paralysis and generalized disease, whereas the human strain is less pathogenic with little dissemination from the brain. A difference in reaction in nerve tissue between the bovine and human types has been observed by many workers investigating tuberculous meningitis.

Bertrand and his co-workers inoculated rabbits with a suspension containing one-twentieth milligram of the growth of tubercle bacilli in pure culture and observed that organisms of the bovine type induced a paralysis which began in seven to fourteen days after the injection and progressed until the death of the animal on from the twentieth to the thirtieth day. At autopsy such animals had local brain lesions and manifested the dissemination of the bovine type organisms by extensive lung and visceral lesions. Human strains caused no sign of disease, and only traces of scar tissue could be observed at the site of inoculation when the animals were killed and examined.

We inoculated a series of rabbits in an attempt to apply this method to the type differentiation of cultures obtained from sputum specimens. The relatively early appearance of symptoms, as described by Bertrand, Bablet, and Bloch, led us to hope that the method would be a more economical one for typing than that involved in the intravenous inoculation of rabbits where more than one rabbit must be inoculated and observed for a month or more.

Suspensions of the growth of *Mycobacterium tuberculosis* on Petragnani's medium were prepared by macerating the cultures in isotonic salt solution with a glass rod in a conical tube. The suspensions were standardized to the opacity of a previously determined scale so that 0.2 c.c. contained one-twentieth milligram of organisms. This dose (0.2 c.c.) was inoculated intracerebrally through the squamoparietal suture, using a tuberculin syringe and a 20 gauge hypodermic needle cut to 1 cm. in length.

Known bovine strains were first inoculated in order to observe the differentiating reaction as described by Bertrand, Bablet, and Bloch. Two of these bovine type cultures were obtained from the National Type Collection, the third had been recently isolated from a lesion in a bull, and the fourth had been isolated from a bone lesion in a child. All had been tested for type by intravenous

*From the Department of Hygiene, University of California.
Aided by a grant from the Research Board, University of California.
Received for publication, January 11, 1939.

inoculation of rabbits. Eight rabbits were inoculated with the four strains. In from ten to twenty days these animals showed paralysis, which progressed until they died from ten to twenty-five days later. All were emaciated and completely paralyzed. At autopsy the dura and the dorsal surface of the hemisphere of the brain into which the inoculation had been made were filled with tubercles. In all but one rabbit tubercles were demonstrated in the lungs. In another animal tubercles were observed in the liver and kidney as well. In these animals the incubation period and the course of the disease were longer than those reported by Bertrand, Bablet, and Bloch. This may be accounted for by the fact that the animals used by us weighed approximately 4 kg. and may have been larger than those used by the French workers, who did not note the size or age of rabbit they used.

TABLE I

INTRACEREBRAL INJECTIONS OF CULTURES OF MYCOBACTERIUM TUBERCULOSIS

NUMBER OF CULTURES INJECTED	NUMBER OF RABBITS REACTION	NUMBER OF RABBITS NO REACTION	TOTAL
31		31	31
12	12		12
5	5	5	10
2	4		4
11		22	22
Total 61	21	58	79

After we had observed the reaction of known bovine strains, we injected 61 cultures of *Mycobacterium tuberculosis* isolated from sputum intracerebrally into 79 rabbits (Table I). Nineteen of these cultures killed 21 rabbits. These animals exhibited the same symptoms described by Bertrand, Bablet, and Bloch that we had observed in animals inoculated with bovine strains.

Eighteen of our group of 61 cultures were each inoculated into two rabbits. Eleven of these did not produce reactions in either animal; two killed both animals with manifestations characteristic of bovine type infection; five induced such symptoms on first injection but failed to affect the animals when again injected intracerebrally.

The rabbits which presented evidence of bovine infection after intracerebral injection of cultures, as described by Bertrand, Bablet, and Bloch, became paralyzed in from ten to fifty-eight days. These animals were killed in extremis or died from ten to seventy days later. The extended period of paralysis was due to the fact that the animal caretaker fed some of the animals by hand, thus keeping them alive for a longer period of time than the others. In general, however, the period of incubation and the duration of illness were longer than that observed by Bertrand, Bablet, and Bloch. These differences were not due to the age or size of the animals, for young rabbits weighing 2 to 3 kg. were used. Fourteen of these showed definite tubercles in the brain, and acid-fast organisms were demonstrated in smear preparations in the remainder even though macroscopic lesions were not visible. In only 10 of the 21 animals were lung or visceral lesions noted, and these were few in number and small in contrast to the many large tubercles observed in the lungs of the animals inoculated with known bovine strains.

Thirteen of the cultures which had produced symptoms of bovine type infection when injected intracerebrally did not cause any reaction in animals when injected intravenously according to the accepted method (0.1 mg.). Ten additional strains were inoculated both intravenously and intracerebrally but failed to induce any reaction when introduced by either route.

The 58 rabbits injected with the remaining 42 cultures did not show any symptoms of disease during a three-month observation period. Fifty-one of the 58 animals were killed and examined after three months. At autopsy macroscopic tuberculous lesions on the surface of the brain or in the ventricle were apparent in 18 of this group of animals. In many instances the presence of tubercle bacilli in the brain substance was demonstrated even though no macroscopic lesions were visible.

From the results of these experiments we are forced to conclude that the reaction of the bovine strain of *Mycobacterium tuberculosis*, when inoculated into the brain of rabbits, is not unique since 19 strains, obviously human in origin and proved to be the human type by intravenous inoculation, induced symptoms like those produced by known bovine strains and described by Bertrand, Bablet, and Bloch. It would appear to be unwise to discard hastily the classic technique of intravenous injection in order to differentiate the bovine from the human type of *Mycobacterium tuberculosis*.

REFERENCES

1. Bertrand, I., Bablet, J., and Bloch, F.: Sur la determination du type original des souches de bacilles tuberculeux par l'inoculation intracerebrale experimentale, Compt. rend. Soc. de biol. **124**: 108, 1937.
2. Boquet, A., and Broca, R.: Recherches experimentales sur la Meningite Tuberculeuse, Ann. Inst. Pasteur **55**: 8, 1935.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

LEVULOSE TOLERANCE TEST, With Special Reference to Normal Standards, Herbert, F. K. Brit. M. J. 29: 867, 1939.

As a result of earlier work it has been shown that the levulose-tolerance test becomes more reliable if the results are based on estimations of blood levulose rather than total blood sugar.

The dose of levulose is 50 gm. for an adult, and 1 gm. per kg. of body weight for a child. It is given after a night's fast. Samples of venous blood, about 5 c.c., are taken before the dose of levulose, and at half an hour, one hour, and two hours after. Potassium oxalate is used as an anticoagulant; an excess of oxalate should be avoided.

Reagents.—

1. 10 per cent $ZnSO_4 \cdot 7H_2O$.
2. 0.5 N NaOH.
3. Stock diphenylamine solution: 20 per cent diphenylamine in alcohol.
4. Acid-alcohol-diphenylamine reagent: ethyl alcohol, 70 parts; concentrated HCl, 50 parts; stock diphenylamine solution, 6 parts. This reagent should not be more than a month old when used.
5. Stock levulose solution, 1 per cent in saturated benzoic acid. The levulose should be desiccated before weighing.
6. Standard levulose solutions (freshly prepared):
   (a) 1 c.c. stock solution diluted to 200 c.c. with water (3 c.c. = 0.1 mg. levulose).
   (b) 25 c.c. of standard (a) diluted to 100 c.c. (2 c.c. = 0.025 mg. levulose).

Procedure.—Take 2 c.c. plasma in a 15 c.c. centrifuge tube. Add 2 c.c. water and 2 c.c. 10 per cent zinc sulfate solution. Close the tube with a rubber stopper and shake thoroughly. Add 2 c.c. 0.5 N NaOH and again shake thoroughly. Centrifuge the mixture well and pour the supernatant fluid through a small filter (Whatman No. 42 paper, 5.5 cm.). The yield of filtrate is just over 4 c.c. which allows duplicate analyses. In a pyrex test tube (about ⅝ by 6 inches), graduated at 10 c.c., take 2 c.c. filtrate and 6 c.c. acid-alcohol-diphenylamine reagent. The standard levulose solutions (2 c.c. of each standard) are similarly treated. Place the tubes in a boiling water bath for exactly fifteen minutes and then cool them rapidly in running water. Dilute with alcohol to the 10 c.c. mark, and compare the colors in the colorimeter, using standard (b) for comparison with the filtrates from "fasting" plasma and standard (a) for the remainder.

Calculation.—

Let R_s = reading of standard
Let R_u = reading of unknown

Then plasma levulose, mg. per 100 c.c., is given by the formulas:

$\frac{R_s}{R_u}$ by 20 when standard (a) is used;

$\frac{R_s}{R_u}$ by 5 when standard (b) is used.

If absolute figures for blood levulose are required, the total blood sugar is estimated and a correction made for the effect of the blood glucose. The effect of 100 mg. glucose per 100 c.c. is equal to that of 2.4 mg. levulose per 100 c.c.

In performing the levulose-tolerance test in nondiabetic subjects it is justifiable to treat the glucose effect as constant throughout the test and to estimate the blood levulose after the

test dose from the rise above the apparent initial level. For diabetic subjects it is desirable to estimate the total sugar and make the correction, but even in diabetes the effect of the correction on the interpretation of the results is very small. The figures for plasma are identical with those for whole blood.

LEUKOCYTE COUNTS, Serial, Practical Value in the Management of Pulmonary Tuberculosis, Elwood, B. J., and DeCecio, T. Am. Rev. Tubere. **39**: 641, 1939.

A study of leucocytic formulas was made with a view to establish their limits of practicability in an otherwise careful management of pulmonary tuberculosis. It was found that:

1. The evaluation of the initial leucocytic formula discloses that the neutrophilic and total leucocytic levels are significant when elevated of an exudative or cavernous lesion or both. The lymphocytic and monocytic levels and proportions are not significant for clinical interpretation. The application of this knowledge to the initial clinical estimation of tuberculosis conceivably merits consideration.

2. An analysis was made of the clinically significant alterations in the leucocytic formulas during the known course of specific pathologic changes. In instances indicating regressive anatomic trends, no clinically interpretable changes in the leucocytic formula could be found. With the occurrence of progression of disease, as indicated by spread, excavation, or formation of effusion (empyema?), a significant tendency toward the development or maintenance of a neutrophilic leucocytosis was observed. Differential characteristics for the specific nature or form in which disease advances or activity takes place could not be established. A careful correlation of clinical and roentgenologic findings disclosed instances in which the intrinsic value of the blood picture might have asserted itself. Nevertheless a pathologic concept may be drawn which, for immediate clinical purposes, is limited to the recognition of a simple trend in the blood picture to portend or confirm activity or advancement of disease.

3. A wide test of the validity of a more detailed concept of the significance of leucocytic changes found a concurrence with established standards in 61 per cent of several hundred instances of pathologic changes. When individual anatomic trends were considered, a more significant and clinically applicable incidence of compliance of blood counts was noted with the occurrence of such progressive phases as spread, cavitation, or effusion. On the contrary, contradictory or nonsignificant formulas were largely noted in the various expressions of retrogressing lesions. Attempts to find a yield of significant compliance in more detailed pathologic trends were even less successful. The broad application of concepts advanced for the interpretation of leucocyte counts, however scientifically sound, finds insufficient clinical correlation.

4. Accuracy of the clinicoroentgenologic estimate of prognosis occurs in an impressive majority and exceeds significantly that based on serial leucocyte counts. The results of application to prognosis bear out consistently the limitation of the blood picture interpretation of advancing pulmonary tuberculosis.

MENINGITIS, Pneumococcic, Treatment of, With Sulfanilamide, Hewell, B. A., and Mitchell, A. G. J. A. M. A. **112**: 1033, 1939.

There are at least 30 reported cases of recovery from pneumococcic meningitis, including 3 observed by the authors, in which part of the treatment consisted in the use of sulfanilamide or related compounds. It appears reasonable to conclude that sulfanilamide compounds were responsible for these recoveries in most instances, since the mortality rate was so high with other forms of treatment. In a few instances the dose was so small that the effect of the drug must be considered doubtful. It is to be noted that recovery occurred with the use of different compounds, such as prontosil, sulfanilamide, and sulfapyridine; that is to say, the data now available do not permit conclusions concerning the relative merit of these different compounds in the treatment of pneumococcic meningitis.

There are at least 8 cases of pneumococcic meningitis, including 3 observed by the authors, in which sulfanilamide was given and in which recovery did not follow.

It could not be shown statistically that the age of the patient, the dose of sulfanilamide employed, or the type of pneumococcus had any relation to recovery.

Of the patients receiving sulfanilamide who recovered, only one showed pneumococci in the blood culture; 16 other patients had negative blood cultures; and for 13, cultures were not reported. Of the 8 patients receiving sulfanilamide who died, the blood cultures were positive for 7. This indicates that even with the use of sulfanilamide a blood stream infection with pneumococci is a factor in mortality and perhaps also that with such infection large doses of sulfanilamide should be tried.

AMYLOIDOSIS, Renal, Altnow, H. O., Van Winkle, C. C., and Cohen, S. S. Arch. Int. Med. 63: 249, 1939.

The clinical course in 57 cases of renal amyloidosis occurring in tuberculous patients in whom amyloid deposits were demonstrated in microscopic section of renal tissue stained with methyl violet at post-mortem examination is presented.

Tuberculous enteritis was the clinical complication occurring most often in association with renal amyloidosis. If only pathologically verified cases are considered, the incidence of tuberculous enteritis was approximately 60 per cent. If amyloid infiltration of the intestine is added (which includes all cases in which there was a pathologic basis for the symptoms), the incidence was 70 per cent. If cases in which the diagnosis was not verified at necropsy are added, enteritis was present in 82 per cent. Thirty-six patients (65 per cent) showed tuberculous pleuritis, pericarditis, or peritonitis alone or in combination.

A suppurating focus was present in 18 patients (exclusive of patients with pulmonary cavitation).

The date of the presumptive or definite onset (or both) could be determined with reasonable accuracy in 46 patients. An outstanding clinical event or complication that might be associated with the onset of renal amyloidosis occurred in 25 patients (54 per cent). Such clinical events, complications, or conditions were the onset of enteritis, pleural effusion, empyema, development of other suppurating foci, institution of pneumothorax, and other surgical procedures.

Ten patients showed no albuminuria. The average duration of albuminuria when present was twenty-five months, the median being fifteen months. The average duration of heavy albuminuria was fifteen and eight-tenths months, the median being six months. Albuminuria preceded cylindruria in 12 patients.

Fifteen patients showed no cylindruria. The average total duration of cylindruria when present was twenty-one and one-half months, the median being twelve months. The average total duration of heavy cylindruria was nineteen and three-tenths months, the median being seven months. Cylindruria preceded albuminuria in 6 instances.

Urine with a low specific gravity was the most constant manifestation of impaired renal function. It occurred in 4 of every 5 cases. It was an early finding and, contrasted with chronic nephritis, was not associated with nitrogen retention.

Impaired concentration was a frequent finding, as shown by the Volhard test performed on 12 patients. A diminished four-hour output and impaired dilution occurred with decreasing frequency. The intramuscular phenolsulfonephthalein test gave normal results in 12 of 24 patients. Only 5 of 29 patients for whom nonprotein nitrogen determinations for the blood were obtained showed a nitrogen retention of 50 mg. or more per 100 c.c.

Hypertension, according to our data, occupies an inconspicuous place in the clinical picture of renal amyloidosis. It may be incidental rather than related to cause and effect.

Arteriosclerosis of the retinal vessels is present even less often than hypertension. Retinitis was not observed in 19 patients examined. The group examined were patients with outstanding clinical symptoms.

It is approximately correct to say that one-fourth of the patients with renal amyloidosis have edema some time during the early and middle periods of the disease, and two-thirds have terminal edema. There is no agreement between the loss of albumin via the kidney and the edema.

ANEMIA, Aplastic, With Special Reference to the Significance of the Small Lymphocytes, Jordan, H. E. Arch. Path. 27: 1, 1939.

An effort has been made to apply the concept of the lymphocyte as an immature polyvalent cell in an interpretation of the histologic observations on the hemopoietic tissues of a patient who died with clinically typical "aplastic" hypoplastic anemia. The lymph nodes, spleen, and bone marrow were considered as a unit. These three tissues had essentially the same lymphoid structure. They had an essentially uniform appearance of hypoplasia. The lymph nodes and spleen showed evidence of almost complete exhaustion following a period of excessive activity. The predominating cell in the three tissues was the small lymphocyte. Large and medium-sized lymphocytes were relatively more abundant in the bone marrow. The small lymphocytes grew to become large lymphocytes which, as hemocytoblasts, differentiated into erythrocytes within the venous sinuses and into granulocytes in the intervascular stroma. The evidence is in complete accord with the claim that at least one important function of the lymphocytes is to serve as ancestors for erythrocytes and granulocytes. The marrow practically lacked megakaryocytes. It contained many basophilic granulocytes, interpretable as abortive eosinophiles. Both spleen and marrow contained considerable numbers of plasma cells, interpretable as abortive erythroblasts, and many small, pale, disintegrating normoblasts.

VITAMIN C, Ascorbic Acid in Blood and Urine After Oral Administration of a Test Dose of, Goldsmith, G. A., and Ellinger, G. F. Arch. Int. Med. 63: 533, 1939.

The state of vitamin C nutrition may be determined by measuring the excretion of ascorbic acid in the urine for six hours after the administration of 600 mg. of this substance. An alternative procedure consists in the determination of the amount of reduced ascorbic acid in the blood during fasting and at the end of one hour and of three hours after the administration of a similar test dose.

There is a definite relationship between the level of reduced ascrobic acid in the blood and the amount of this substance excreted by the kidneys; when the former exceeds 1.4 mg. per 100 c.c., urinary excretion increases.

Mild vitamin C deficiency is extremely prevalent, and too much importance should not be attached to depletion of the ascorbic acid content of the body in any disease.

INTRAVENOUS THERAPY, Cause of Chills Following, Nelson, C. M. J. A. M. A. 112: 1304, 1939.

Certain factors commonly thought to be influential in producing reactions to venoclysis are eliminated.

Another organism, *B. alcaligenes faecalis*, is added to the list of those known to cause reactions. At the same time *Monilia albicans* is eliminated.

Reactions to hypodermoclysis have value as a simple means of determining the presence or absence of pyrogen in the ordinary saline solution used in hospitals.

Further proof is added in support of the already proved statement that reactions to venoclysis are the result of bacterial contamination of the distilled water used in the preparation of the solutions.

MALARIAL INFECTIONS, Influence of, on the Wassermann and Kahn Reactions, Kitchen, S. F., Webb, E. L., and Kupper, W. H. J. A. M. A. 112: 1443, 1939.

A systematic study of the Wassermann and Kahn reactions was made before, during, and after twenty-five naturally induced attacks of malaria in nonsyphilitic patients. Positive reactions were obtained in every case in which malaria developed clinically. Two

patients showed no positive results from the complement-fixation tests, and two others showed no positive Kahn reactions.

There was an instance of malaria provoking positive serologic reactions and a considerably increased cell count in the cerebrospinal fluid.

Seventy-two per cent of the positive reactions made their first appearance during the third and fourth weeks following inoculation. The first positive reactions were observed both before and after the period of clinical activity in a few instances, but in 68 per cent of the attacks they occurred within the first two weeks of the febrile period. The duration of the "seropositive" period exceeded three weeks in 60 per cent of the cases and extended beyond four weeks in 48 per cent. During this time 320 positive and 307 negative serologic reactions were obtained.

When the results from all tests were totaled, there was observed a tendency for vivax infections to induce a greater proportion of positive serologic results than *P. falciparum* infections. Likewise, the relative number of positive reactions was higher among females with malaria than among males, and among persons up to 34 years of age than among those over that age.

The percentage of positive reactions was highest during the period from fifteen to twenty-one days after the last previous paroxysm.

PREGNANCY, The Visscher-Bowman Test for, Krieger, V. I. M. J. Australia **1**: 463, 1939.

From the observations of the author correlated with the reports of other workers, it is evident that the Visscher-Bowman reaction is not an indication of the presence of the hormone of the anterior lobe of the pituitary in urine of pregnant women. The reaction may be due (a) to the presence of reducing carbohydrates, for example, glucose, particularly in the presence of albumin; (b) to marked concentrations of urinary pigment, with greater possibility of positive reaction in the presence of albumin; and (c) to the presence of other reducing substances in urine.

The Visscher-Bowman test for pregnancy does not reveal the presence of the hormones of the anterior lobe of the pituitary present in the urine of pregnant women.

ENDOCARDITIS, Bacterial, Nature of Vegetations of, Allen, A. C. Arch. Path. **27**: 661, 1939.

The structure of the vegetations in 24 patients with acute and subacute bacterial endocarditis was studied with the aid of differential connective tissue stains.

In agreement with current descriptions, the bulk of each of these vegetations was found generally to consist of a necrotic zone beneath a layer of bacteria.

Isolated clumps, fragmented strands, and segmented bits of typical adult elastic and collagenous tissue, easily distinguished from granulation tissue or fibrin, were found scattered through this necrotic zone in both acute and subacute vegetations. These fibers are considered evidence of a destructive, rather than of a reparative, process.

The question as to whether or not this necrotic zone is really essentially a platelet mass, in accordance with the current concept, is discussed on the basis of morphologic observations.

It is concluded, contrary to the long-established current concept, that the bulk of a vegetation of acute or subacute bacterial endocarditis is derived from components of inflamed and fibroplastically deformed valves and is not derived from blood flowing over the valves.

REVIEWS

Die elektrischen Gruppen in Biologie und Medizin*

THIS new pamphlet of Rudolph Keller, successful newspaper man, for whom biology is a hobby, will again fascinate the reader, even the most scientifically trained biologist. Starting with the concept that the electropolarity of tissues and of substances is a dominating factor in all biological activities, Keller summarizes the literature concerning facts demonstrating such polarity in various biological problems, including diseases and pharmacologic effects. Many of the phenomena presented are well known under other names such as ionantagonism, basic and acid dyes, vital staining, irreciprocal permeability, endosmotic capillary activity, cataphoresis, etc. But Keller links them all together by his concept in a very interesting way and points out various important future research problems.—*Ernst Fischer.*

Clinics on Secondary Gastro-Intestinal Disorders†

THIS book represents a group of selected clinics intended to emphasize that gastro-intestinal symptoms often reflect abnormalities in other organs.

While this may appear to be a trite observation, there is ample evidence that it is not always remembered in actual practice. This volume, therefore, should be of value to the student and the practicing physician to whom it is primarily addressed.

It is, indeed, a practical book which all may well read profitably.

Avian Tuberculosis Infection‡

AS THE author says in the preface to this book: "The ability of the bacillus of tuberculosis of chickens to infect a long list of heterologous hosts makes this member of the genus *Mycobacterium* of considerable interest to the student of infectious diseases. . . . To attempt to provide within a single volume a source where information concerning questions relating to the avian tubercle bacillus will be available, has been the motive for the preparation of this monograph."

With this purpose in view, Professor Feldman has prepared a comprehensive and authoritative review which, beyond question, is at present the standard text on this subject destined to remain a standard reference for years to come.

The book is well organized, well written, well illustrated, and well printed. In its thirteen chapters will be found a well-oriented and critically analyzed discussion of all that is known and pertinent to the subject. The book is of interest and value not only to the veterinarian and those engaged in public health, but also to the research worker, the pathologist, and those interested in tuberculosis in all its phases.

---

*Die elektrischen Gruppen in Biologie und Medizin. By Rudolph Keller. 92 pages (including 14 pages of English summary and 7 pages quotation in English). Sperber-Verlag, Zurich, 1938.

†Clinics on Secondary Gastro-Intestinal Disorders; Reciprocal Relationships. By Julius Friedenwald, M.D., Professor Emeritus of Gastroenterology, University of Maryland; Theodore H. Morrison, M.D., Clinical Professor of Gastroenterology, University of Maryland; and Samuel Morrison, M.D., Assistant Professor of Gastroenterology, University of Maryland. Cloth, 251 pages, $3.00. William Wood & Co., Baltimore, Md.

‡Avian Tuberculosis Infections. By William H. Feldman, D.V.M., M.S., Associate in Division of Experimental Medicine, Institute of Experimental Medicine; Associate Professor of Comparative Pathology, Mayo Foundation for Medical Education and Research, Graduate School, University of Minnesota. Cloth, 483 pages, 109 figures, $7.00. Williams & Wilkins Co., Baltimore, Md.

Index photographed at the
beginning for the convenience
of the microfilm user.